Title: Hazards of diisobutyl phthalate (DIBP) exposure: A systematic review of animal toxicology studies

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ED_012964_00000438-00001

ABSTRACT

Background: Biomonitoring studies indicate a trend towards increased human exposure to diisobutyl phthalate (DIBP), a replacement for dibutyl phthalate (DBP). Recent systematic reviews have found DIBP to be a male reproductive toxicant, but have not evaluated other hazards of DIBP exposure.
Objective: To inform chemical risk assessment, we performed a systematic review to identify and characterize outcomes within six broad hazard categories (male reproductive, female reproductive, developmental, liver, kidney, and cancer) following exposure of nonhuman mammalian animals to DIBP or the primary metabolite, monoisobutyl phthalate (MIBP).
Methods: A literature search was conducted in four online scientific databases (PubMed, Web of Science, Toxline, and TSCATS2), and augmented by review of regulatory sources as well as forward and backward searches. Studies were identified for inclusion based on defined PECO (Population, Exposure, Comparator, Outcome) criteria. Studies were evaluated using criteria defined a priori for reporting quality, risk of bias, and sensitivity using a domain-based approach. Evidence was synthesized by outcome and life stage of exposure, and strength of evidence was summarized into categories of *robust*, *moderate*, *slight*, *indeterminate*, or *compelling evidence of no effect,* using a structured framework.
Results: Nineteen toxicological studies in rats or mice met the inclusion criteria. There was *robust* evidence that DIBP causes male reproductive toxicity. Male rats and mice exposed to DIBP during gestation had decreased testosterone and adverse effects on sperm or testicular histology, with additional phthalate syndrome effects observed in male rats. There was also evidence of androgen-dependent and -independent male reproductive effects in rats and mice following peripubertal or young adult exposure to DIBP or MIBP, but confidence was reduced because of concerns over risk of bias and sensitivity in the available studies. There was also *robust* evidence that DIBP causes developmental toxicity; specifically, increased post-implantation loss and decreased pre- and postnatal growth. For other hazards, evidence was limited by the small number of studies, experimental designs that were suboptimal for evaluating outcomes, and study evaluation concerns such as incomplete reporting of methods and results. There was *slight* evidence for female reproductive effects, and *indeterminate* evidence for liver, kidney, and cancer.
Conclusion: Results support DIBP as a children's health concern and indicate that male reproductive and developmental toxicities are hazards of DIBP exposure, with slight evidence for female reproductive toxicity. Data gaps include the need for more studies on male reproductive effects following postnatal and adult exposure, and studies to characterize potential hormonal mechanisms in females.

ED_012964_00000438-00002

1. INTRODUCTION:

Diisobutyl phthalate (DIBP) is a member of the phthalate ester class of chemicals and is used as a plasticizer to provide flexibility and durability to a wide variety of industrial and consumer products, including paints, lacquers, printing ink, pulp and paper, carpet, concrete, nail polish, and cosmetics [ADDIN EN.CITE <EndNote><Cite><Author>HSDB</Author><Year>2017</Year><RecNum>55</RecNum><DisplayText>(HSDB 2017)</DisplayText><record><rec-number>55</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1518910494">55</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>HSDB,</author></authors></contributors><titles><title>Diisobutyl phthalate</title></titles><dates><year>2017</year></dates><pub-location>Bethesda, MD</pub-location><publisher>National Library of Medicine</publisher><label>680311</label><urls><related-urls><url>https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@DOCNO+5247</url></related-urls></urls></record></Cite></EndNote>]. Because of its use in household products, people are exposed to DIBP via food and indoor environments [ADDIN EN.CITE <EndNote><Cite><Author>Wormuth</Author><Year>2006</Year><RecNum>2</RecNum><DisplayText>(Wormuth et al. 2006)</DisplayText><record><rec-number>2</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460009">2</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wormuth, M.</author><author>Scheringer, M.</author><author>Vollenweider, M.</author><author>Hungerbuhler, K.</author></authors></contributors><titles><title>What are the sources of exposure to eight frequently used phthalic acid esters in Europeans?</title><secondary-title>Risk Analysis</secondary-title><alt-title>Risk Anal</alt-title></titles><periodical><full-title>Risk Analysis</full-title><abbr-1>Risk Anal</abbr-1></periodical><alt-periodical><full-title>Risk Analysis</full-title><abbr-1>Risk Anal</abbr-1></alt-periodical><pages>803-824</pages><volume>26</volume><number>3</number><dates><year>2006</year></dates><isbn>ISSN 0272-4332
EISSN 1539-6924</isbn><accession-num>16834635</accession-num><label>680214</label><urls><related-urls><url>http://dx.doi.org/10.1111/j.1539-6924.2006.00770.</url></related-urls></urls><electronic-resource-num>10.1111/j.1539-6924.2006.00770.</electronic-resource-num><language>English</language></record></Cite></EndNote>]. DIBP is readily absorbed via oral ingestion [ADDIN EN.CITE <EndNote><Cite><Author>Koch</Author><Year>2012</Year><RecNum>57</RecNum><DisplayText>(Koch et al. 2012)</DisplayText><record><rec-number>57</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519055415">57</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Koch, H. M.</author><author>Christensen, K. L. Y.</author><author>Harth, V.</author><author>Lorber, M.</author><author>Brüning, T.</author></authors></contributors><titles><title>Di-n-butyl phthalate (DnBP) and diisobutyl phthalate (DiBP) metabolism in a human volunteer after single oral doses</title><secondary-title>Archives of Toxicology</secondary-title><alt-title>Arch Toxicol</alt-title></titles><periodical><full-title>Archives of Toxicology</full-title><abbr-1>Arch Toxicol</abbr-1></periodical><alt-periodical><full-title>Archives of Toxicology</full-title><abbr-1>Arch Toxicol</abbr-

1></alt-periodical><pages>1829-
1839</pages><volume>86</volume><number>12</number><dates><year>2012</year></dates><isbn>
ISSN 0340-5761
EISSN 1432-0738</isbn><accession-num>22820759</accession-
num><label>1311698</label><urls><related-urls><url>http://dx.doi.org/10.1007/s00204-012-0908-
1</url></related-urls></urls><electronic-resource-num>10.1007/s00204-012-0908-1</electronic-
resource-num><language>English</language></record></Cite></EndNote>] and dermal exposure [
ADDIN EN.CITE
<EndNote><Cite><Author>Elsisi</Author><Year>1989</Year><RecNum>56</RecNum><DisplayText>(Elsisi et al. 1989)</DisplayText><record><rec-number>56</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519055137">56</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Elsisi, A.
E.</author><author>Carter, D. E.</author><author>Sipes, I.
G.</author></authors></contributors><titles><title>Dermal absorption of phthalate diesters in
rats</title><secondary-title>Fundamental and Applied Toxicology</secondary-title><alt-title>Fundam
Appl Toxicol</alt-title></titles><periodical><full-title>Fundamental and Applied Toxicology</full-
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6832</isbn><accession-num>2925020</accession-num><label>675074</label><urls><related-
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num>10.1093/toxsci/12.1.70</electronic-resource-
num><language>English</language></record></Cite></EndNote>], and is rapidly hydrolyzed to its
primary metabolite, monoisobutyl phthalate (MIBP). DIBP and MIBP are distributed systemically in
blood [ADDIN EN.CITE ADDIN EN.CITE.DATA], and there is evidence these chemicals can be
transferred to human breast milk [ADDIN EN.CITE ADDIN EN.CITE.DATA] and cross the placental
barrier [ADDIN EN.CITE
<EndNote><Cite><Author>Wittassek</Author><Year>2009</Year><RecNum>61</RecNum><DisplayText>(Wittassek et al. 2009)</DisplayText><record><rec-number>61</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps"
timestamp="1519056639">61</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wittassek, M.</author><author>Angerer,
J.</author><author>Kolossa-Gehring, M.</author><author>Schafer, S.
D.</author><author>Klockenbusch, W.</author><author>Dobler, L.</author><author>Gunsel, A.
K.</author><author>Muller, A.</author><author>Wiesmuller, G.
A.
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study</title><secondary-title>International Journal of Hygiene and Environmental Health</secondary-
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498</pages><volume>212</volume><number>5</number><dates><year>2009</year></dates><isbn>I
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Biomonitoring studies indicate that DIBP exposures have increased in recent years, possibly because of DIBP being used as a substitute for other phthalates such as dibutyl phthalate (DBP) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. For instance, data from the National Health and Nutrition Examination Survey (NHANES) indicate that the detection frequency of MIBP in urine increased from 72% of the U.S. general population in 2001-2002 to 96% in 2009-2010. Over this period, urinary concentrations of MIBP increased monotonically, while the metabolites of DBP and several other phthalates decreased [ADDIN EN.CITE <EndNote><Cite><Author>Zota</Author><Year>2014</Year><RecNum>63</RecNum><DisplayText>(Zota et al. 2014)</DisplayText><record><rec-number>63</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519056746">63</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Zota, A. R.</author><author>Calafat, A. M.</author><author>Woodruff, T. J.</author></authors></contributors><titles><title>Temporal trends in phthalate exposures: findings from the national health and nutrition examination survey, 2001-2010</title><secondary-title>Environmental Health Perspectives</secondary-title><alt-title>Environ Health Perspect</alt-title></titles><periodical><full-title>Environmental Health Perspectives</full-title><abbr-1>Environ Health Perspect</abbr-1></periodical><alt-periodical><full-title>Environmental Health Perspectives</full-title><abbr-1>Environ Health Perspect</abbr-1></alt-periodical><pages>235-241</pages><volume>122</volume><number>3</number><dates><year>2014</year></dates><isbn>ISSN 0091-6765
EISSN 1552-9924</isbn><accession-num>24425099</accession-num><label>2241689</label><urls><related-urls><url>http://dx.doi.org/10.1289/ehp.1306681</url></related-urls></urls><electronic-resource-num>10.1289/ehp.1306681</electronic-resource-num><language>English</language></record></Cite></EndNote>].

Because DIBP was historically used less compared to other phthalates, it has also been relatively less studied. However, recent systematic reviews have characterized DIBP as a male reproductive toxicant [ADDIN EN.CITE <EndNote><Cite><Author>CHAP</Author><Year>2014</Year><RecNum>4</RecNum><DisplayText>(CHAP 2014; NAS 2017)</DisplayText><record><rec-number>4</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460456">4</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>CHAP,</author></authors></contributors><titles><title>Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with appendices)</title></titles><dates><year>2014</year></dates><pub-location>Bethesda, MD</pub-location><publisher>U.S. Consumer Product Safety Commission, Directorate for Health Sciences</publisher><label>2439960</label><urls><related-urls><url>http://www.cpsc.gov/en/Regulations-Laws--Standards/Statutes/The-Consumer-Product-Safety-Improvement-Act/Phthalates/Chronic-Hazard-Advisory-Panel-CHAP-on-Phthalates/</url></related-urls></urls></record></Cite><Cite><Author>NAS</Author><Year>2017</Year><RecNum>47</RecNum>

<record><rec-number>47</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509470742">47</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>NAS,</author></authors></contributors><titles><title>Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals</title><secondary-title>Consensus Study Report</secondary-title></titles><dates><year>2017</year></dates><pub-location>Washington, D.C.</pub-location><publisher>The National Academies Press</publisher><label>3982546</label><urls><related-urls><url>http://dx.doi.org/10.17226/24758</url></related-urls></urls><electronic-resource-num>10.17226/24758</electronic-resource-num><language>English</language></record></Cite></EndNote>]. This is a common hazard among many phthalates and is generally the greatest concern associated with phthalate exposure. In rats, in utero exposure to phthalates during the critical window of male sexual differentiation produces a phenotype known as “phthalate syndrome”, which is characterized by underdevelopment of male reproductive organs, decreased anogenital distance (AGD), female-like nipple retention, cryptorchidism, and germ cell toxicity [ADDIN EN.CITE ADDIN EN.CITE.DATA]. These effects can be causally linked to decreased testicular production of androgens, which are integral to male sexual development; decreased insulin-like-3 (INSL3) hormone, which regulates transabdominal testicular descent; and direct targeting of seminiferous cord formation, Sertoli cells, and germ cell development via an unknown mode of action (MOA) that is independent of effects on androgen production [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Based on recommendations following a systematic review by a Chronic Hazard Advisory Panel (CHAP) [ADDIN EN.CITE <EndNote><Cite><Author>CHAP</Author><Year>2014</Year><RecNum>4</RecNum><DisplayText>(CHAP 2014)</DisplayText><record><rec-number>4</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460456">4</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>CHAP,</author></authors></contributors><titles><title>Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with appendices)</title></titles><dates><year>2014</year></dates><pub-location>Bethesda, MD</pub-location><publisher>U.S. Consumer Product Safety Commission, Directorate for Health Sciences</publisher><label>2439960</label><urls><related-urls><url>http://www.cpsc.gov/en/Regulations-Laws--Standards/Statutes/The-Consumer-Product-Safety-Improvement-Act/Phthalates/Chronic-Hazard-Advisory-Panel-CHAP-on-Phthalates/</url></related-urls></urls></record></Cite></EndNote>], which evaluated the effects on children’s health of all phthalates and phthalate alternatives used in children’s toys and child care articles, DIBP is one of eight phthalates that the U.S. Consumer Products Safety Commission (CPSC) has permanently banned from children’s toys and care articles at any amount greater than 0.1% (16 CFR § 1307 2017). The CHAP stated that, although DIBP is not widely used in children’s toys, it shares a similar anti-androgenic MOA with other banned phthalates, and therefore may contribute to cumulative risks to children. Additionally, the National Academy of Sciences (NAS) recently conducted a systematic review on phthalates and male reproductive tract development with the purpose of evaluating the potential for low-dose toxicity. The NAS concluded that DIBP is a presumed human health hazard based on dose-related effects on testosterone (T) production in animal studies [ADDIN EN.CITE

<EndNote><Cite><Author>NAS</Author><Year>2017</Year><RecNum>47</RecNum><DisplayText>(NAS 2017)</DisplayText><record><rec-number>47</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509470742">47</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>NAS,</author></authors></contributors><titles><title>Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals</title><secondary-title>Consensus Study Report</secondary-title></titles><dates><year>2017</year></dates><pub-location>Washington, D.C.</pub-location><publisher>The National Academies Press</publisher><label>3982546</label><urls><related-urls><url>http://dx.doi.org/10.17226/24758</url></related-urls></urls><electronic-resource-num>10.17226/24758</electronic-resource-num><language>English</language></record></Cite></EndNote>].

Systematic review methods have only recently been applied for the purposes of chemical risk assessment, but offer the advantages of being focused, objective, and transparent. The [ADDIN EN.CITE <EndNote><Cite ExcludeAuth="1" ExcludeYear="1"><Author>CHAP</Author><Year>2014</Year><RecNum>4</RecNum><record><rec-number>4</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460456">4</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>CHAP,</author></authors></contributors><titles><title>Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with appendices)</title></titles><dates><year>2014</year></dates><pub-location>Bethesda, MD</pub-location><publisher>U.S. Consumer Product Safety Commission, Directorate for Health Sciences</publisher><label>2439960</label><urls><related-urls><url>http://www.cpsc.gov/en/Regulations-Laws--Standards/Statutes/The-Consumer-Product-Safety-Improvement-Act/Phthalates/Chronic-Hazard-Advisory-Panel-CHAP-on-Phthalates/</url></related-urls></urls></record></Cite><Cite AuthorYear="1"><Author>CHAP</Author><Year>2014</Year><RecNum>4</RecNum><record><rec-number>4</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460456">4</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>CHAP,</author></authors></contributors><titles><title>Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with appendices)</title></titles><dates><year>2014</year></dates><pub-location>Bethesda, MD</pub-location><publisher>U.S. Consumer Product Safety Commission, Directorate for Health Sciences</publisher><label>2439960</label><urls><related-urls><url>http://www.cpsc.gov/en/Regulations-Laws--Standards/Statutes/The-Consumer-Product-Safety-Improvement-Act/Phthalates/Chronic-Hazard-Advisory-Panel-CHAP-on-Phthalates/</url></related-urls></urls></record></Cite></EndNote>]CHAP [ADDIN EN.CITE <EndNote><Cite ExcludeAuth="1"><Author>CHAP</Author><Year>2014</Year><RecNum>4</RecNum><DisplayText>(2014)</DisplayText><record><rec-number>4</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460456">4</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>CHAP,</author></authors></contributors><titles><title>Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with

appendices)</title></titles><dates><year>2014</year></dates><pub-location>Bethesda, MD</pub-location><publisher>U.S. Consumer Product Safety Commission, Directorate for Health Sciences</publisher><label>2439960</label><urls><related-urls><url>http://www.cpsc.gov/en/Regulations-Laws--Standards/Statutes/The-Consumer-Product-Safety-Improvement-Act/Phthalates/Chronic-Hazard-Advisory-Panel-CHAP-on-Phthalates/</url></related-urls></urls></record></Cite></EndNote>] and [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>NAS</Author><Year>2017</Year><RecNum>47</RecNum><DisplayText>NAS (2017)</DisplayText><record><rec-number>47</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509470742">47</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>NAS,</author></authors></contributors><titles><title>Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals</title><secondary-title>Consensus Study Report</secondary-title></titles><dates><year>2017</year></dates><pub-location>Washington, D.C.</pub-location><publisher>The National Academies Press</publisher><label>3982546</label><urls><related-urls><url>http://dx.doi.org/10.17226/24758</url></related-urls></urls><electronic-resource-num>10.17226/24758</electronic-resource-num><language>English</language></record></Cite></EndNote>] systematic reviews of phthalates both focused on evaluating epidemiological and toxicological studies with male reproductive outcomes, of which the NAS focused specifically on three phthalate syndrome endpoints (T, anogenital distance, and hypospadias) after gestational exposure. These systematic reviews did not evaluate other hazards caused by phthalate exposure, such as female reproductive effects or effects in other organ systems.

To gain a more comprehensive understanding of the spectrum of effects after DIBP exposure, we performed a systematic review of the animal toxicology literature for DIBP for six broad hazard categories (male reproductive, female reproductive, developmental, liver, kidney, cancer) that have been commonly associated with phthalate exposure.

2. METHODS:

This systematic review is part of a larger evaluation of health effects after exposure to multiple phthalates. The literature searches and screening, study evaluation, data extraction, and evidence synthesis methods are described in detail in the systematic review protocol (Supplemental Materials) and summarized here. The systematic review protocol also provides detailed definitions for the terminology used to describe study evaluation and evidence synthesis, which are summarized in Figure 1.

2.1. Literature searches and screening

A literature search was conducted in four online scientific databases (PubMed, Web of Science, Toxline, and TSCATS2), using search terms designed to capture all potentially pertinent studies. Initial database searches were conducted in February 2013, with updates performed every 6-12 months through July 2017. The results of this literature search were supplemented by forward and backward searches, searching citations from key references, manual search of citations from key regulatory documents, and by addition of references that had been previously identified from an earlier DIBP review effort and added to EPA's Health and Environmental Research Online (HERO) database.

A PECO (Population, Exposure, Comparator, Outcome) was developed to frame the research question and guide the screening of relevant studies. The PECO identifies the following as the inclusion criteria for the systematic review of DIBP animal toxicology studies (see protocol for the full PECO):

- Population: Nonhuman mammalian animal species (whole organism) of any life stage.
- Exposure: Any administered dose of DIBP or MIBP as singular compounds, via oral, dermal, or inhalation routes of exposure.
- Comparator: Exposure to vehicle-only or untreated control
- Outcome: Any examination of male reproductive, female reproductive, developmental, liver, kidney, or cancer outcomes.

Title/abstract and full text screening was performed by two reviewers, and all identified animal toxicology studies underwent full-text screening to determine compliance with the PECO. Peer-reviewed studies that contained original data and complied with the PECO were selected for inclusion, and were moved forward for study evaluation. Studies providing supporting health effects data (e.g. mechanistic, genotoxic, or toxicokinetic studies) were also compiled in HERO and annotated during the screening process ([HYPERLINK "https://hero.epa.gov/hero/index.cfm/project/page/project_id/2320"]).

2.2. Study evaluation

For each study selected for inclusion, the quality and informativeness of the evidence was rated by evaluating for metrics related to reporting quality, risk of bias, and sensitivity. Reporting quality refers to how well the study authors communicated the details of the methods and results. Risk of bias, sometimes referred to as internal validity, is the extent to which the design or conduct of a study may alter the ability to provide accurate (unbiased) evidence to support the relationship between exposure and effects [ADDIN EN.CITE
<EndNote><Cite><Author>Higgins</Author><Year>2011</Year><RecNum>286</RecNum><DisplayText>(Higgins 2011)</DisplayText><record><rec-number>286</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1522683248">286</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Higgins, J.P.T.; Green, S.</author></authors></contributors><titles><title>Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. Chapter 8: Assessing Risk of Bias in Included Studies. The Cochrane Collaboration [updated March 2011].
</title></titles><dates><year>2011</year></dates><urls><related-urls><url>www.cochranehandbook.org</url></related-urls></urls></record></Cite></EndNote>]. Sensitivity refers to the extent to which a study is likely to detect a true effect caused by exposure [ADDIN EN.CITE
<EndNote><Cite><Author>Cooper</Author><Year>2016</Year><RecNum>32</RecNum><DisplayText>(Cooper et al. 2016)</DisplayText><record><rec-number>32</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509464913">32</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Cooper, G.</author><author>Lunn, R.</author><author>Agerstrand, M.</author><author>Glenn, B.</author><author>Kraft, A.</author><author>Luke, A.</author><author>Ratcliffe, J.</author></authors></contributors><titles><title>Study sensitivity: Evaluating the ability to detect effects in systematic reviews of chemical exposures</title><secondary-title>Environment

International</secondary-title><alt-title>Environ Int</alt-title></titles><periodical><full-title>Environment International</full-title><abbr-1>Environ Int</abbr-1></periodical><alt-periodical><full-title>Environment International</full-title><abbr-1>Environ Int</abbr-1></alt-periodical><pages>605-610</pages><volume>92-93</volume><dates><year>2016</year></dates><isbn>ISSN 0160-4120
EISSN 1873-6750</isbn><label>3121908</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.envint.2016.03.017</url></related-urls></urls><electronic-resource-num>10.1016/j.envint.2016.03.017</electronic-resource-num><language>English</language></record></Cite></EndNote>].

Study evaluation was conducted for the following domains: reporting quality, selection or performance bias, confounding/variable control, reporting or attrition bias, exposure methods sensitivity, and outcome measures and results display. Within each domain, reviewers considered one or more metrics designed to identify any potential limitations of the study. For instance, within the "exposure methods sensitivity" domain, metrics were 1) characterization of exposure to the compound of interest and 2) utility of the exposure design for the endpoint of interest. At least two reviewers independently assessed each study, and any conflicts were resolved through discussion among reviewers or other technical experts~~the chemical assessment team~~. When information critical to the evaluation was missing from a study, an attempt was made to contact the study authors for clarification.

For each study, for each of the study evaluation metrics, reviewers reached a consensus on a rating of *Good*, *Adequate*, *Poor*, or *Critically Deficient*. These individual ratings were then combined to reach an overall study confidence classification of *High*, *Medium*, *Low*, or *Uninformative*. The evaluation process was performed separately for each outcome reported in a study, as the utility of a study may vary for different outcomes. A discussion of outcome-specific study evaluation considerations encountered over the course of this systematic review is provided in [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Dishaw</Author><Year>, in preparation</Year><RecNum>290</RecNum><DisplayText>Dishaw et al. (, in preparation)</DisplayText><record><rec-number>290</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1523359294">290</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Dishaw, L.</author><author>Arzuaga, X.</author><author>Yost, E.</author><author>Kraft, A.</author><author>Luke, A.</author><author>Walker, T.</author><author>Thayer, K.</author></authors></contributors><titles><title>Applying study evaluation strategies in the systematic review of animal toxicology studies for chemical risk assessment: A case study for phthalates</title></titles><dates><year>, in preparation</year></dates><urls></urls></record></Cite></EndNote>]. All study evaluation ratings are documented and publicly available ~~on the page for this assessment~~ in Health Assessment Workspace Collaborative (HAWC), a free and open source web-based software application ([HYPERLINK "https://hawcprd.epa.gov/assessment/497/"]).

2.3. Data extraction

Data from included studies were extracted into HAWC ([HYPERLINK "https://hawcprd.epa.gov/assessment/497/"]). Dose levels are presented as mg/kg-day. For dietary exposure studies, dose conversions to mg/kg-day were made using US EPA default food or water

consumption rates and body weights for the species/strain and sex of the animal of interest [ADDIN EN.CITE <EndNote><Cite><Author>US EPA</Author><Year>1988</Year><RecNum>15</RecNum><DisplayText>(US EPA 1988)</DisplayText><record><rec-number>15</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509461391">15</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>US EPA,</author></authors></contributors><titles><title>Recommendations for and documentation of biological values for use in risk assessment</title></titles><pages>1-395</pages><dates><year>1988</year></dates><pub-location>Cincinnati, OH</pub-location><publisher>U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment</publisher><isbn>EPA/600/6-87/008</isbn><label>64560</label><urls><related-urls><url>http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855</url></related-urls></urls><language>English</language></record></Cite></EndNote>].

2.4. Evidence synthesis

Data were synthesized and evaluated according to the age and developmental stage of exposure to account for life stage-specific windows of susceptibility, as recommended by the [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>US EPA</Author><Year>2006</Year><RecNum>50</RecNum><DisplayText>US EPA (2006)</DisplayText><record><rec-number>50</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1510870631">50</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>US EPA,</author></authors></contributors><titles><title>A framework for assessing health risk of environmental exposures to children</title></titles><pages>1-145</pages><dates><year>2006</year></dates><pub-location>Washington, DC</pub-location><publisher>U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment</publisher><isbn>EPA/600/R-05/093F</isbn><label>194567</label><urls><related-urls><url>http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363</url></related-urls></urls><language>English</language></record></Cite></EndNote>]. For each outcome, at each life stage of exposure, the available evidence from the included animal studies was synthesized using a narrative approach, using the following considerations to articulate the strengths and weaknesses of the available evidence [adapted from [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Hill</Author><Year>1965</Year><RecNum>46</RecNum><DisplayText>Hill (1965)</DisplayText><record><rec-number>46</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509466265">46</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hill, A. B.</author></authors></contributors><titles><title>The environment and disease: Association or causation?</title><secondary-title>Proceedings of the Royal Society of Medicine</secondary-title><alt-title>Proc R Soc Med</alt-title></titles><periodical><full-title>Proceedings of the Royal Society of Medicine</full-title><abbr-1>Proc R Soc Med</abbr-1></periodical><alt-periodical><full-title>Proceedings of the Royal Society of Medicine</full-title><abbr-1>Proc R Soc Med</abbr-1></alt-periodical><pages>295-

300</pages><volume>58</volume><number>5</number><dates><year>1965</year></dates><isbn>ISSN 0035-9157</isbn><accession-num>14283879</accession-num><label>71664</label><urls></urls><language>English</language></record></Cite></EndNote>]]: consistency, biological gradient (dose-response), strength (effect magnitude) and precision, biological plausibility, and coherence. When available, informative mechanistic data were used to augment the qualitative syntheses. Based on this synthesis, each outcome was assigned a strength of evidence conclusion of *Robust*, *Moderate*, *Slight*, *Indeterminate, or Compelling evidence of no effect*. *Robust* and *Moderate* describe evidence that supports a hazard, differentiated by the quantity and quality of information available to rule out alternative explanations for the results. *Slight* evidence includes situations in which there is some evidence that supports a hazard but a conclusion of *Moderate* does not apply. *Indeterminate* describes a situation where there are no studies available for that evidence stream or the evidence is inconsistent and cannot provide a basis for making a conclusion in either direction. *Compelling evidence of no effect* represents a situation where extensive evidence across a range of populations and exposures identified no association. The ratings for individual outcomes were then summarized into an overall strength of evidence conclusion for each of the six hazards (male reproductive, female reproductive, developmental, liver, kidney, cancer). Rationales for strength of evidence conclusions are presented in evidence profile tables using a structured format based on the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach for evaluating certainty in the evidence [ADDIN EN.CITE ADDIN EN.CITE.DATA].

3. RESULTS

3.1. Study selection

Literature search and screening results are summarized in Figure 2. The literature search retrieved a total of 1,184 unique records for DIBP, of which 31 were identified as animal toxicology studies during title/abstract screening and proceeded to full text screening. Of these, 25 met the defined PECO criteria for inclusion and were moved forward for study evaluation. The animal toxicology studies that did not meet PECO criteria consisted of three studies that used intraperitoneal injection [ADDIN EN.CITE ADDIN EN.CITE.DATA], two studies in nonmammalian species [chickens: [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Choy</Author><Year>1975</Year><RecNum>68</RecNum><DisplayText>Choy (1975)</DisplayText><record><rec-number>68</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058505">68</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Choy, I.</author></authors><translated-authors><author>Apex, Translations</author></translated-authors></contributors><titles><title>Effects of sulfathiazole, phthalylsulfathiazole and phthalic acid esters on the development of chick embryos [Translated]</title><translated-title>ÜBer die beeinflussung von sulfathiazol, phthalylsulfathiazol und phthal-sÄureestern auf die entwicklung des hÜhnerembryos</translated-title></titles><pages>187-201</pages><dates><year>1975</year></dates><label>3035664</label><urls></urls><language>English</language></record></Cite></EndNote>]; zebrafish: [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Sohn</Author><Year>2016</Year><RecNum>69</RecNum><DisplayText>Sohn et al. (2016)</DisplayText><record><rec-number>69</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058544">69</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sohn,

J.</author><author>Kim, S.</author><author>Koschorreck, J.</author><author>Kho, Y.</author><author>Choi, K.</author></authors></contributors><titles><title>Alteration of sex hormone levels and steroidogenic pathway by several low molecular weight phthalates and their metabolites in male zebrafish (Danio rerio) and/or human adrenal cell (H295R) line</title><secondary-title>Journal of Hazardous Materials</secondary-title><alt-title>J Hazard Mater</alt-title></titles><periodical><full-title>Journal of Hazardous Materials</full-title><abbr-1>J Hazard Mater</abbr-1></periodical><alt-periodical><full-title>Journal of Hazardous Materials</full-title><abbr-1>J Hazard Mater</abbr-1></alt-periodical><pages>45-54</pages><volume>320</volume><dates><year>2016</year></dates><isbn>ISSN 0304-3894
EISSN 1873-3336</isbn><accession-num>27513369</accession-num><label>3469480</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.jhazmat.2016.08.008</url></related-urls></urls><electronic-resource-num>10.1016/j.jhazmat.2016.08.008</electronic-resource-num><language>English</language></record></Cite></EndNote>]], and one behavioral study in mice [ADDIN EN.CITE <EndNote><Cite><Author>Ma</Author><Year>2013</Year><RecNum>70</RecNum><DisplayText>(Ma et al. 2013)</DisplayText><record><rec-number>70</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058609">70</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Ma, N.</author><author>Liu, S.</author><author>Gao, P.</author><author>Cao, P.</author><author>Xu, H.</author></authors><translated-authors><author>Trusted Translations, Inc</author></translated-authors></contributors><titles><title>Effect of diisobutyl phthalate on learning and memory behavior and apoptosis of hippocampus cells in mice [Translated]</title><secondary-title>Wei Sheng Yan Jiu [Journal of Hygiene Research]</secondary-title><alt-title>Wei Sheng Yan Jiu</alt-title><translated-title><style face="normal" font="default" charset="134" size="100%">邻苯二甲酸二异丁酯对小鼠认知行为及海马神经细胞凋亡的影响</style></translated-title></titles><periodical><full-title>Wei Sheng Yan Jiu [Journal of Hygiene Research]</full-title><abbr-1>Wei Sheng Yan Jiu</abbr-1></periodical><alt-periodical><full-title>Wei Sheng Yan Jiu [Journal of Hygiene Research]</full-title><abbr-1>Wei Sheng Yan Jiu</abbr-1></alt-periodical><pages>57-60</pages><volume>42</volume><number>1</number><dates><year>2013</year></dates><isbn>ISSN 1000-8020</isbn><accession-num>23596708</accession-num><label>2349624</label><urls></urls><language>English</language></record></Cite></EndNote>]. Additionally, the included article by [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>University of Rochester</Author><Year>1954</Year><RecNum>72</RecNum><DisplayText>University of Rochester (1954)</DisplayText><record><rec-number>72</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058725">72</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>University of Rochester,</author></authors></contributors><titles><title>Preliminary acute toxicity tests and short term feeding tests of rats and dogs given di-isobutyl phthalate and di-butyl phthalate</title></titles><dates><year>1954</year></dates><pub-location>Rochester, NY</pub-location><publisher>University of Rochester School of Medicine and Dentistry</publisher><isbn>EPA/OTS Doc #878210833</isbn><label>680305</label><work-type>TSCA

Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0205995</url></related-urls></urls><language>English</language></record></Cite></EndNote>] presented data from multiple experiments, including a rat study that complied with the PECO and was moved forward for study evaluation, and a dog study that was rejected for not having a control group.

At the study evaluation phase, one article was excluded from further consideration because of critical deficiencies in reporting [ADDIN EN.CITE <EndNote><Cite><Author>Eastman Kodak</Author><Year>1954</Year><RecNum>73</RecNum><DisplayText>(Eastman Kodak 1954)</DisplayText><record><rec-number>73</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058827">73</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Eastman Kodak,</author></authors></contributors><titles><title>Screening test on the toxicity of diisobutyl phthalate</title></titles><dates><year>1954</year></dates><pub-location>Rochester, NY</pub-location><isbn>EPA/OTS Doc #878214403</isbn><label>680271</label><work-type>TSCA Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0206525</url></related-urls></urls><language>English</language></record></Cite></EndNote>], and four articles (all of which presented data from the same reproductive toxicity study in rats) were excluded because they tested a single high dose of DIBP (4000 mg/kg-day) that caused a high rate of mortality in the exposed dams [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Two of the included articles [ADDIN EN.CITE ADDIN EN.CITE.DATA] presented data from the same set of experiments.[1] Therefore, a total of 20 articles presenting data from 19 unique studies were included in this analysis (Table 1).

3.2. Summary of included studies

Table 1 summarizes the experimental designs and outcomes evaluated in the included studies. All were oral exposures (gavage or diet) of rats or mice. Ten were prenatal developmental toxicity studies that dosed pregnant dams with DIBP; of these, nine exposed rats during gestation only, and one exposed mice either during gestation only or from gestation through weaning (via lactational exposure). The remaining nine studies were postnatal-only exposures of weanling or peripubertal rats or mice to DIBP or MIBP.

Most of the gestational exposure studies were designed with the primary objective of evaluating male reproductive effects in F1 offspring, although two rat studies focused on fetal survival and external, skeletal, and soft tissue/visceral malformations and variations [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Because of the focus on male reproductive effects, four of the rat studies exposed animals only during the critical window of male sexual differentiation [ADDIN EN.CITE ADDIN EN.CITE.DATA], which is approximately between gestation days (GDs) 14-18. Other studies exposed pregnant dams for longer durations, beginning immediately after mating [ADDIN EN.CITE <EndNote><Cite><Author>Wang</Author><Year>2017</Year><RecNum>80</RecNum><Prefix>mice: </Prefix><DisplayText>(mice: Wang et al. 2017)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps"

[1] Here, we cite Foster et al. 1981 (a peer-reviewed publication) for testis weights and histopathology, and Foster et al. 1982 (an edited book) for liver and kidney weights from the same study.

timestamp="1519059737">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, X.</author><author>Sheng, N.</author><author>Cui, R.</author><author>Zhang, H.</author><author>Wang, J.</author><author>Dai, J.</author></authors></contributors><titles><title>Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring</title><secondary-title>Chemosphere</secondary-title><alt-title>Chemosphere</alt-title></titles><periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></periodical><alt-periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></alt-periodical><pages>260-267</pages><volume>172</volume><dates><year>2017</year></dates><isbn>ISSN 0045-6535
EISSN 1879-1298</isbn><accession-num>28081510</accession-num><label>3483278</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.chemosphere.2017.01.011</url></related-urls></urls><electronic-resource-num>10.1016/j.chemosphere.2017.01.011</electronic-resource-num><language>English</language></record></Cite></EndNote>], within a few days after implantation [ADDIN EN.CITE ADDIN EN.CITE.DATA], or at mid-gestation [ADDIN EN.CITE <EndNote><Cite><Author>Saillenfait</Author><Year>2008</Year><RecNum>82</RecNum><Prefix>rats: </Prefix><DisplayText>(rats: Saillenfait et al. 2008)</DisplayText><record><rec-number>82</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059943">82</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><pages>107-115</pages><volume>26</volume><number>2</number><dates><year>2008</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>18706996</accession-num><label>680390</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2008.07.006</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2008.07.006</electronic-resource-num><language>English</language></record></Cite></EndNote>], and continuing through the remainder of gestation. One study in rats [ADDIN EN.CITE <EndNote><Cite><Author>Saillenfait</Author><Year>2008</Year><RecNum>82</RecNum><DisplayText>(Saillenfait et al. 2008)</DisplayText><record><rec-number>82</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059943">82</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><pages>107-

115</pages><volume>26</volume><number>2</number><dates><year>2008</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>18706996</accession-num><label>680390</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2008.07.006</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2008.07.006</electronic-resource-num><language>English</language></record></Cite></EndNote>] and one in mice [ADDIN EN.CITE <EndNote><Cite><Author>Wang</Author><Year>2017</Year><RecNum>80</RecNum><DisplayText>(Wang et al. 2017)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059737">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, X.</author><author>Sheng, N.</author><author>Cui, R.</author><author>Zhang, H.</author><author>Wang, J.</author><author>Dai, J.</author></authors></contributors><titles><title>Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring</title><secondary-title>Chemosphere</secondary-title><alt-title>Chemosphere</alt-title></titles><periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></periodical><alt-periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></alt-periodical><pages>260-267</pages><volume>172</volume><dates><year>2017</year></dates><isbn>ISSN 0045-6535
EISSN 1879-1298</isbn><accession-num>28081510</accession-num><label>3483278</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.chemosphere.2017.01.011</url></related-urls></urls><electronic-resource-num>10.1016/j.chemosphere.2017.01.011</electronic-resource-num><language>English</language></record></Cite></EndNote>] allowed dams to give birth and evaluated male reproductive effects in adult F1 offspring, while the remaining rat studies sacrificed dams near the end of gestation and evaluated effects in fetuses. Regardless of the primary objective of the study, all gestational exposure studies provided relevant data on F1 offspring survival, growth, and/or maternal reproductive outcomes.

Six of the postnatal exposure studies exposed sexually immature male rats or mice of varying ages (weanling to peripubertal) for up to 7 days and focused on male reproductive outcomes [ADDIN EN.CITE ADDIN EN.CITE.DATA]. One study by Sedha et al. (2015) focused on estrogenic outcomes in weanling female rats. Two studies by the University of Rochester [ADDIN EN.CITE <EndNote><Cite ExcludeAuth="1"><Author>University of Rochester</Author><Year>1953</Year><RecNum>71</RecNum><DisplayText>(1953, 1954)</DisplayText><record><rec-number>71</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058725">71</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>University of Rochester,</author></authors></contributors><titles><title>One month feeding tests of di-isobutyl phthalate with cover letter</title></titles><dates><year>1953</year></dates><pub-location>Rochester, NY</pub-location><publisher>University of Rochester School of Medicine and Dentistry</publisher><isbn>EPA/OTS Doc #878212229</isbn><label>680304</label><work-type>TSCA Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0205995</url></related-urls></urls><language>English</language></record></Cite><Cite ExcludeAuth="1"><Author>University of

Rochester</Author><Year>1954</Year><RecNum>72</RecNum><record><rec-number>72</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058725">72</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>University of Rochester,</author></authors></contributors><titles><title>Preliminary acute toxicity tests and short term feeding tests of rats and dogs given di-isobutyl phthalate and di-butyl phthalate</title></titles><dates><year>1954</year></dates><pub-location>Rochester, NY</pub-location><publisher>University of Rochester School of Medicine and Dentistry</publisher><isbn>EPA/OTS Doc #878210833</isbn><label>680305</label><work-type>TSCA Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0205995</url></related-urls></urls><language>English</language></record></Cite></EndNote>] exposed male or female rats for 1 or 4 months beginning at weaning and focused on general toxicity. All reported information on postnatal growth, and most also reported information on liver and kidney effects.

None of the gestational or postnatal exposure studies provided information on cancer.

3.3. Study evaluation

Overall study confidence classifications by outcome are summarized in Table 1, with detailed evaluations available in HAWC ([HYPERLINK "https://hawcprd.epa.gov/assessment/497/"]). Among the gestational exposure study outcomes, confidence was generally *high*, although some study outcomes were rated *medium* or *low confidence* because of specific concerns. For instance, the rat gestational exposure studies by [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Furr</Author><Year>2014</Year><RecNum>87</RecNum><DisplayText>Furr et al. (2014)</DisplayText><record><rec-number>87</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060024">87</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Furr, J. R.</author><author>Lambright, C. S.</author><author>Wilson, V. S.</author><author>Foster, P. M.</author><author>Gray, L. E., Jr.</author></authors></contributors><titles><title>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</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>403-424</pages><volume>140</volume><number>2</number><dates><year>2014</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>24798384</accession-num><label>2510906</label><urls><related-urls><url>http://dx.doi.org/10.1093/toxsci/kfu081</url></related-urls></urls><electronic-resource-num>10.1093/toxsci/kfu081</electronic-resource-num><language>English</language></record></Cite></EndNote>] and Hannas et al. [ADDIN EN.CITE ADDIN EN.CITE.DATA] used a relatively small sample size (generally n=3-4 dams/treatment group) that authors stated did not provide the statistical power to consistently detect anything but rather large alterations in maternal weight gain and fetal viability, or decreases in T production of less than 20-25%; our reviewers determined that the T measurements in these studies had adequate sensitivity, but confidence in the maternal weight gain and fetal survival data was reduced because of sensitivity

concerns. For evaluation of maternal toxicity, confidence was reduced in the maternal body weight gain measurements in several studies that did not adjust for gravid uterine weight, the calculation of which is considered preferable because it facilitates the interpretation of maternal toxicity relative to effects on fetal body weight [ADDIN EN.CITE <EndNote><Cite><Author>US EPA</Author><Year>1991</Year><RecNum>19</RecNum><DisplayText>(US EPA 1991)</DisplayText><record><rec-number>19</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509462676">19</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>US EPA,</author></authors></contributors><titles><title>Guidelines for developmental toxicity risk assessment</title></titles><pages>1-71</pages><dates><year>1991</year></dates><pub-location>Washington, DC</pub-location><publisher>U.S. Environmental Protection Agency, Risk Assessment Forum</publisher><isbn>EPA/600/FR-91/001</isbn><label>8567</label><urls><related-urls><url>http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23162</url></related-urls></urls><language>English</language></record></Cite></EndNote>]. Additionally, in the only available gestational exposure study in mice [ADDIN EN.CITE <EndNote><Cite><Author>Wang</Author><Year>2017</Year><RecNum>80</RecNum><DisplayText>(Wang et al. 2017)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059737">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, X.</author><author>Sheng, N.</author><author>Cui, R.</author><author>Zhang, H.</author><author>Wang, J.</author><author>Dai, J.</author></authors></contributors><titles><title>Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring</title><secondary-title>Chemosphere</secondary-title><alt-title>Chemosphere</alt-title></titles><periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></periodical><alt-periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></alt-periodical><pages>260-267</pages><volume>172</volume><dates><year>2017</year></dates><isbn>ISSN 0045-6535
EISSN 1879-1298</isbn><accession-num>28081510</accession-num><label>3483278</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.chemosphere.2017.01.011</url></related-urls></urls><electronic-resource-num>10.1016/j.chemosphere.2017.01.011</electronic-resource-num><language>English</language></record></Cite></EndNote>], confidence in male reproductive, body weight, and organ weight data was reduced because data were presented as an average of individual pups, rather than using the litter as the experimental unit. Failure to account for litter effects has the potential to overestimate the statistical significance of experimental findings [ADDIN EN.CITE ADDIN EN.CITE.DATA]. All rat gestational exposure studies used the litter as the experimental unit for presentation and analysis of F1 offspring data.

All outcomes reported in the postnatal exposure studies were rated as *medium* or *low confidence*. In general, these studies had incomplete reporting of experimental designs and results, which led to concerns for risk of bias and sensitivity. Reporting deficiencies included lack of information about the age or pubertal status of the animals at the time of exposure, strain of the animals, sample size, or methods used to allocate test animals to experimental groups, and presentation of only qualitative (rather than quantitative) results. For evaluation of male reproductive effects, confidence was reduced in studies that presented only relative testis weight (as a percentage of body weight) without presenting

absolute testis weight; it has been shown that testis weights are not modeled well by an organ-to-body weight ratio because testis and body weights are not proportional [ADDIN EN.CITE <EndNote><Cite><Author>Bailey</Author><Year>2004</Year><RecNum>102</RecNum><DisplayText>(Bailey et al. 2004)</DisplayText><record><rec-number>102</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519066625">102</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bailey, S. A.</author><author>Zidell, R. H.</author><author>Perry, R. W.</author></authors></contributors><titles><title>Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint?</title><secondary-title>Toxicologic Pathology</secondary-title><alt-title>Toxicol Pathol</alt-title></titles><periodical><full-title>Toxicologic Pathology</full-title><abbr-1>Toxicol Pathol</abbr-1></periodical><alt-periodical><full-title>Toxicologic Pathology</full-title><abbr-1>Toxicol Pathol</abbr-1></alt-periodical><pages>448-466</pages><volume>32</volume><number>4</number><dates><year>2004</year></dates><isbn>ISSN 0192-6233
EISSN 1533-1601</isbn><accession-num>15204968</accession-num><label>782883</label><urls><related-urls><url>http://dx.doi.org/10.1080/01926230490465874</url></related-urls></urls><electronic-resource-num>10.1080/01926230490465874</electronic-resource-num><language>English</language></record></Cite></EndNote>], so this may be a less sensitive measurement compared to absolute testis weight.

3.4. Male reproductive effects

Figures indicating the doses at which statistically significant male reproductive effects occurred are provided in Supplemental Materials (Figures S1 – S3). Morphological and histopathological effects in F1 postnatal and adult males from the multi-dose study by [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Saillenfait</Author><Year>2008</Year><RecNum>82</RecNum><DisplayText>Saillenfait et al. (2008)</DisplayText><record><rec-number>82</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059943">82</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><pages>107-115</pages><volume>26</volume><number>2</number><dates><year>2008</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>18706996</accession-num><label>680390</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2008.07.006</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2008.07.006</electronic-resource-num><language>English</language></record></Cite></EndNote>] are summarized in Figures S4 and S5, respectively.

3.4.1. Summary of gestational (F1) exposure studies

A dose-related decrease in androgens was observed in male offspring from all rat and mouse gestational exposure studies that evaluated this outcome. This includes six studies that evaluated fetal testicular T production or testicular T or androstenedione (AN) levels in fetal rats [ADDIN EN.CITE ADDIN EN.CITE.DATA], and one study that evaluated serum and testicular T levels in adult mice that had been exposed during gestation and/or through weaning [ADDIN EN.CITE <EndNote><Cite><Author>Wang</Author><Year>2017</Year><RecNum>80</RecNum><DisplayText>(Wang et al. 2017)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059737">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, X.</author><author>Sheng, N.</author><author>Cui, R.</author><author>Zhang, H.</author><author>Wang, J.</author><author>Dai, J.</author></authors></contributors><titles><title>Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring</title><secondary-title>Chemosphere</secondary-title><alt-title>Chemosphere</alt-title></titles><periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></periodical><alt-periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></alt-periodical><pages>260-267</pages><volume>172</volume><dates><year>2017</year></dates><isbn>ISSN 0045-6535
EISSN 1879-1298</isbn><accession-num>28081510</accession-num><label>3483278</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.chemosphere.2017.01.011</url></related-urls></urls><electronic-resource-num>10.1016/j.chemosphere.2017.01.011</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Across these studies, changes in T were statistically significant at doses as low as 200 mg/kg-day, and the magnitude of effect was often large (decreased up to 96% compared to controls). Several of these studies also reported decreased testicular expression of genes or proteins in the steroidogenesis pathway in rats [ADDIN EN.CITE ADDIN EN.CITE.DATA] and in mice [ADDIN EN.CITE <EndNote><Cite><Author>Wang</Author><Year>2017</Year><RecNum>80</RecNum><DisplayText>(Wang et al. 2017)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059737">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, X.</author><author>Sheng, N.</author><author>Cui, R.</author><author>Zhang, H.</author><author>Wang, J.</author><author>Dai, J.</author></authors></contributors><titles><title>Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring</title><secondary-title>Chemosphere</secondary-title><alt-title>Chemosphere</alt-title></titles><periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></periodical><alt-periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></alt-periodical><pages>260-267</pages><volume>172</volume><dates><year>2017</year></dates><isbn>ISSN 0045-6535
EISSN 1879-1298</isbn><accession-num>28081510</accession-num><label>3483278</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.chemosphere.2017.01.011</url></related-urls></urls><electronic-resource-num>10.1016/j.chemosphere.2017.01.011</electronic-resource-num><language>English</language></record></Cite></EndNote>], providing mechanistic evidence in both species to corroborate the observed decrease in fetal testicular testosterone. The affected genes

ED_012964_00000438-00020

or proteins included steroid acute regulatory protein (StAR) and scavenger receptor class B type 1 (SR-B1), which are involved in cholesterol uptake and transport; and 3β-hydroxysteroid dehydrogenase (3β-HSD), cytochrome P450 (CYP) side chain cleavage (P450scc or CYP11A1), and CYP17A1, which are steroidogenic enzymes. [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Hannas</Author><Year>2012</Year><RecNum>86</RecNum><DisplayText>Hannas et al. (2012)</DisplayText><record><rec-number>86</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060024">86</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hannas, B. R.</author><author>Lambright, C. S.</author><author>Furr, J.</author><author>Evans, N.</author><author>Foster, P. M. D.</author><author>Gray, E. L.</author><author>Wilson, V. S.</author></authors></contributors><titles><title>Genomic biomarkers of phthalate-induced male reproductive developmental toxicity: A targeted RT-PCR array approach for defining relative potency</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>544-557</pages><volume>125</volume><number>2</number><dates><year>2012</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>22112501</accession-num><label>1004932</label><urls><related-urls><url>http://dx.doi.org/10.1093/toxsci/kfr315</url></related-urls></urls><electronic-resource-num>10.1093/toxsci/kfr315</electronic-resource-num><language>English</language></record></Cite></EndNote>] also reported decreased testicular gene expression of INSL3 in rats. Overall, the evidence for decreased fetal testicular T production after gestational exposure was found to be *robust*.

In postnatal and adult male rats that had been exposed during the critical window of male sexual differentiation during gestation, [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Saillenfait</Author><Year>2008</Year><RecNum>82</RecNum><DisplayText>Saillenfait et al. (2008)</DisplayText><record><rec-number>82</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059943">82</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><pages>107-115</pages><volume>26</volume><number>2</number><dates><year>2008</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>18706996</accession-num><label>680390</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2008.07.006</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2008.07.006</electronic-resource-num><language>English</language></record></Cite></EndNote>] reported numerous dose-related

outcomes that are consistent with decreased testosterone and INSL3: decreased AGD, increased time to puberty (preputial separation), nipple retention, increased rate of external malformations of the reproductive tract (hypospadias, exposed os penis, cryptorchidism[2], cleft prepuce; Figure S4), and decreased reproductive organ weights (testis, epididymides, seminal vesicles, prostate). Other gestational exposure studies that evaluated fetal rats also observed decreased male AGD [ADDIN EN.CITE <EndNote><Cite><Author>Borch</Author><Year>2006</Year><RecNum>88</RecNum><DisplayText>(Borch et al. 2006)</DisplayText><record><rec-number>88</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060127">88</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Borch, J.</author><author>Axelstad, M.</author><author>Vinggaard, A. M.</author><author>Dalgaard, M.</author></authors></contributors><titles><title>Diisobutyl phthalate has comparable anti-androgenic effects to di-n-butyl phthalate in fetal rat testis</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>183-190</pages><volume>163</volume><number>3</number><dates><year>2006</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>16458459</accession-num><label>674974</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.toxlet.2005.10.020</url></related-urls></urls><electronic-resource-num>10.1016/j.toxlet.2005.10.020</electronic-resource-num><language>English</language></record></Cite></EndNote>] and increased incidence of cryptorchidism, with approximately two-thirds of testes in the highest dose group (1000 mg/kg-day) located in the upper half of the abdominal cavity [ADDIN EN.CITE <EndNote><Cite><Author>Saillenfait</Author><Year>2006</Year><RecNum>8</RecNum><DisplayText>(Saillenfait et al. 2006)</DisplayText><record><rec-number>8</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460688">8</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabate, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Developmental toxic effects of diisobutyl phthalate, the methyl-branched analogue of di-n-butyl phthalate, administered by gavage to rats</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>39-46</pages><volume>165</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>16516415</accession-num><label>680389</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.toxlet.2006.01.013</url></related-urls></urls><electronic-resource-num>10.1016/j.toxlet.2006.01.013</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Conversely, in weanling and adult

[2] Cryptorchidism is reported as "nonscrotal testis" in in adult males in Saillenfait et al. 2008 and "ectopic testis" in male fetuses in Saillenfait et al. 2006.

mice, male AGD was not affected and effects on testis weight were inconsistent [ADDIN EN.CITE <EndNote><Cite><Author>Wang</Author><Year>2017</Year><RecNum>80</RecNum><DisplayText>(Wang et al. 2017)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059737">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, X.</author><author>Sheng, N.</author><author>Cui, R.</author><author>Zhang, H.</author><author>Wang, J.</author><author>Dai, J.</author></authors></contributors><titles><title>Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring</title><secondary-title>Chemosphere</secondary-title><alt-title>Chemosphere</alt-title></titles><periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></periodical><alt-periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></alt-periodical><pages>260-267</pages><volume>172</volume><dates><year>2017</year></dates><isbn>ISSN 0045-6535
EISSN 1879-1298</isbn><accession-num>28081510</accession-num><label>3483278</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.chemosphere.2017.01.011</url></related-urls></urls><electronic-resource-num>10.1016/j.chemosphere.2017.01.011</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Overall, based on strength of the data in rats, the evidence for effects on male morphological development after gestational exposure was found to be *robust*, and evidence for effects on male reproductive organ weight was found to be *moderate*.

In these same animals, histopathological analysis found dose-related increases in lesions in the fetal rat testis [ADDIN EN.CITE <EndNote><Cite><Author>Borch</Author><Year>2006</Year><RecNum>88</RecNum><DisplayText>(Borch et al. 2006)</DisplayText><record><rec-number>88</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060127">88</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Borch, J.</author><author>Axelstad, M.</author><author>Vinggaard, A. M.</author><author>Dalgaard, M.</author></authors></contributors><titles><title>Diisobutyl phthalate has comparable anti-androgenic effects to di-n-butyl phthalate in fetal rat testis</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>183-190</pages><volume>163</volume><number>3</number><dates><year>2006</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>16458459</accession-num><label>674974</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.toxlet.2005.10.020</url></related-urls></urls><electronic-resource-num>10.1016/j.toxlet.2005.10.020</electronic-resource-num><language>English</language></record></Cite></EndNote>] and adult rat testis [ADDIN EN.CITE <EndNote><Cite><Author>Saillenfait</Author><Year>2008</Year><RecNum>82</RecNum><DisplayText>(Saillenfait et al. 2008)</DisplayText><record><rec-number>82</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059943">82</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J.

P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><pages>107-115</pages><volume>26</volume><number>2</number><dates><year>2008</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>18706996</accession-num><label>680390</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2008.07.006</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2008.07.006</electronic-resource-num><language>English</language></record></Cite></EndNote>], with oligospermia or total azoospermia[3] in the corresponding epididymides [ADDIN EN.CITE <EndNote><Cite><Author>Saillenfait</Author><Year>2008</Year><RecNum>82</RecNum><Suffix>`, Figure SI-5</Suffix><DisplayText>(Saillenfait et al. 2008, Figure SI-5)</DisplayText><record><rec-number>82</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059943">82</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><pages>107-115</pages><volume>26</volume><number>2</number><dates><year>2008</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>18706996</accession-num><label>680390</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2008.07.006</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2008.07.006</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Adult male mice exposed to DIBP during gestation had decreased sperm concentration and motility, which reached statistical significance in mice that were exposed from gestation through weaning [ADDIN EN.CITE <EndNote><Cite><Author>Wang</Author><Year>2017</Year><RecNum>80</RecNum><DisplayText>(Wang et al. 2017)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059737">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, X.</author><author>Sheng, N.</author><author>Cui, R.</author><author>Zhang, H.</author><author>Wang, J.</author><author>Dai, J.</author></authors></contributors><titles><title>Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring</title><secondary-title>Chemosphere</secondary-title><alt-title>Chemosphere</alt-title></titles><periodical><full-

[3] Azoospermia refers to a lack of spermatozoa, and oligospermia refers to reduced spermatozoa.

title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></periodical><alt-periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></alt-periodical><pages>260-267</pages><volume>172</volume><dates><year>2017</year></dates><isbn>ISSN 0045-6535
EISSN 1879-1298</isbn><accession-num>28081510</accession-num><label>3483278</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.chemosphere.2017.01.011</url></related-urls></urls><electronic-resource-num>10.1016/j.chemosphere.2017.01.011</electronic-resource-num><language>English</language></record></Cite></EndNote>]. These outcomes are consistent with effects on sperm development and function that have been observed for other phthalates, which are thought to be mediated via androgen-dependent and -independent pathways [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Given the exposure-response gradient and consistency across species, the evidence for effects on testicular histology and sperm parameters was found to be *robust*.

3.4.2. Summary of postnatal (weanling or peripubertal) exposure studies

Effects on androgens (measured as testicular T or serum T or dihydrotestosterone [DHT] levels) were inconsistent across a series of studies by Oishi and Hiraga [ADDIN EN.CITE ADDIN EN.CITE.DATA], in which immature (~5-week-old) rats or mice were exposed to DIBP or MIBP in the diet for 7 days. In rats, androgen levels were increased compared to controls [ADDIN EN.CITE ADDIN EN.CITE.DATA], whereas testicular T levels in mice were similar [ADDIN EN.CITE <EndNote><Cite><Author>Oishi</Author><Year>1980</Year><RecNum>91</RecNum><DisplayText>(Oishi and Hiraga 1980c)</DisplayText><record><rec-number>91</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060513">91</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Oishi, S.</author><author>Hiraga, K.</author></authors></contributors><titles><title>Effect of phthalic acid esters on mouse testes</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>413-416</pages><volume>5</volume><number>6</number><dates><year>1980</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>7394838</accession-num><label>680366</label><urls><related-urls><url>http://dx.doi.org/10.1016/0378-4274(80)90024-7</url></related-urls></urls><electronic-resource-num>10.1016/0378-4274(80)90024-7</electronic-resource-num><language>English</language></record></Cite></EndNote>] or significantly decreased [ADDIN EN.CITE <EndNote><Cite><Author>Oishi</Author><Year>1980</Year><RecNum>90</RecNum><DisplayText>(Oishi and Hiraga 1980b)</DisplayText><record><rec-number>90</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060475">90</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Oishi, S.</author><author>Hiraga, K.</author></authors></contributors><titles><title>Effects of phthalic acid monoesters on mouse testes</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-

1></alt-periodical><pages>239-242</pages><volume>6</volume><number>4-5</number><dates><year>1980</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>7423552</accession-num><label>675362</label><urls><related-urls><url>http://dx.doi.org/10.1016/0378-4274(80)90126-5</url></related-urls></urls><electronic-resource-num>10.1016/0378-4274(80)90126-5</electronic-resource-num><language>English</language></record></Cite></EndNote>] compared to controls. Confidence in these outcomes was reduced because the pubertal status of these animals was uncertain; whereas [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Oishi</Author><Year>1980</Year><RecNum>93</RecNum><DisplayText>Oishi and Hiraga (1980a)</DisplayText><record><rec-number>93</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060609">93</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Oishi, S.</author><author>Hiraga, K.</author></authors></contributors><titles><title>Testicular atrophy induced by phthalic acid monoesters: Effects of zinc and testosterone concentrations</title><secondary-title>Toxicology</secondary-title><alt-title>Toxicology</alt-title></titles><periodical><full-title>Toxicology</full-title><abbr-1>Toxicology</abbr-1></periodical><alt-periodical><full-title>Toxicology</full-title><abbr-1>Toxicology</abbr-1></alt-periodical><pages>197-202</pages><volume>15</volume><number>3</number><dates><year>1980</year></dates><isbn>ISSN 0300-483X
EISSN 1879-3185</isbn><accession-num>7466832</accession-num><label>675363</label><urls><related-urls><url>http://dx.doi.org/10.1016/0300-483X(80)90053-0</url></related-urls></urls><electronic-resource-num>10.1016/0300-483X(80)90053-0</electronic-resource-num><language>English</language></record></Cite></EndNote>] reported that the rats had positive sperm counts and were between puberty and sexual maturity, the pubertal status of animals in the other three studies was not reported. Susceptibility to phthalate-induced effects on androgen levels is dependent on the life stage of exposure, with young adults likely being less susceptible than pre-pubertal animals [ADDIN EN.CITE <EndNote><Cite><Author>Arzuaga</Author><Year>, in preparation</Year><RecNum>289</RecNum><DisplayText>(Arzuaga et al., in preparation)</DisplayText><record><rec-number>289</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1523359129">289</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Arzuaga, X.</author><author>Walker, T.</author><author>Cooper, G.</author><author>Hotchkiss, A.</author><author>Yost, E.</author></authors></contributors><titles><title>Application of the adverse outcome pathway (AOP) framework to assess species concordance and human relevance of dibutyl phthalate (DBP)-induced male reproductive toxicity </title></titles><dates><year>, in preparation</year></dates><urls></urls></record></Cite></EndNote>]. The discord in findings could therefore be based on issues such as timing of exposure and timing of assessment. Given these study concerns and the inconsistency in results across studies, the evidence for effects on T was found to be *indeterminate*.

Despite the inconsistent findings for androgen levels, effects on male reproductive organ weight provide support for an androgen-dependent MOA in animals exposed during weanling and/or young adult life stages. A dose-related decrease in absolute and relative testis weight was consistently observed across all rat studies [ADDIN EN.CITE ADDIN EN.CITE.DATA], with no significant effects on prostate or seminal vesicle weight [ADDIN EN.CITE

<EndNote><Cite><Author>Foster</Author><Year>1981</Year><RecNum>77</RecNum><DisplayText>(Foster et al. 1981)</DisplayText><record><rec-number>77</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059055">77</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Foster, P. M.</author><author>Lake, B. G.</author><author>Thomas, L. V.</author><author>Cook, M. W.</author><author>Gangolli, S. D.</author></authors></contributors><titles><title>Testicular effects and zinc excretion produced by various isomers of monobutyl-o-phthalate in the rat</title><secondary-title>Chemico-Biological Interactions</secondary-title><alt-title>Chem Biol Interact</alt-title></titles><periodical><full-title>Chemico-Biological Interactions</full-title><abbr-1>Chem Biol Interact</abbr-1></periodical><alt-periodical><full-title>Chemico-Biological Interactions</full-title><abbr-1>Chem Biol Interact</abbr-1></alt-periodical><pages>233-238</pages><volume>34</volume><number>2</number><dates><year>1981</year></dates><isbn>ISSN 0009-2797
EISSN 1872-7786</isbn><accession-num>7460085</accession-num><label>815876</label><urls><related-urls><url>http://dx.doi.org/10.1016/0009-2797(81)90134-4</url></related-urls></urls><electronic-resource-num>10.1016/0009-2797(81)90134-4</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Decreased absolute testis weights were also observed in 21-day-old mice exposed to DIBP for 7 days [ADDIN EN.CITE <EndNote><Cite><Author>Zhu</Author><Year>2010</Year><RecNum>89</RecNum><DisplayText>(Zhu et al. 2010)</DisplayText><record><rec-number>89</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060268">89</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Zhu, X. B.</author><author>Tay, T. W.</author><author>Andriana, B. B.</author><author>Alam, M. S.</author><author>Choi, E. K.</author><author>Tsunekawa, N.

<author>Kanai, Y.</author><author>Kurohmaru, M.</author></authors></contributors><titles><title>Effects of di-iso-butyl phthalate on testes of prepubertal rats and mice</title><secondary-title>Okajimas Folia Anatomica Japonica</secondary-title><alt-title>Okajimas Folia Anat Jpn</alt-title></titles><periodical><full-title>Okajimas Folia Anatomica Japonica</full-title><abbr-1>Okajimas Folia Anat Jpn</abbr-1></periodical><alt-periodical><full-title>Okajimas Folia Anatomica Japonica</full-title><abbr-1>Okajimas Folia Anat Jpn</abbr-1></alt-periodical><pages>129-136</pages><volume>86</volume><number>4</number><dates><year>2010</year></dates><isbn>ISSN 0030-154X
EISSN 1881-1736</isbn><accession-num>20560449</accession-num><label>673046</label><urls><related-urls><url>http://dx.doi.org/10.2535/ofaj.86.129</url></related-urls></urls><electronic-resource-num>10.2535/ofaj.86.129</electronic-resource-num><language>English</language></record></Cite></EndNote>]

, although statistically significant effects occurred at higher doses compared to rats in the same study. Conversely, 5-week-old mice exposed to DIBP or MIBP for 7 days had a statistically significant increase in relative testis weight compared to control [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Relative testis weight has been found to be an unreliable metric because testis and body weights are not proportional [ADDIN EN.CITE <EndNote><Cite><Author>Bailey</Author><Year>2004</Year><RecNum>102</RecNum><DisplayText>(Bailey et al. 2004)</DisplayText><record><rec-number>102</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519066625">102</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bailey, S. A.</author><author>Zidell, R. H.</author><author>Perry, R.

W.</author></authors></contributors><titles><title>Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint?</title><secondary-title>Toxicologic Pathology</secondary-title><alt-title>Toxicol Pathol</alt-title></titles><periodical><full-title>Toxicologic Pathology</full-title><abbr-1>Toxicol Pathol</abbr-1></periodical><alt-periodical><full-title>Toxicologic Pathology</full-title><abbr-1>Toxicol Pathol</abbr-1></alt-periodical><pages>448-466</pages><volume>32</volume><number>4</number><dates><year>2004</year></dates><isbn>ISSN 0192-6233
EISSN 1533-1601</isbn><accession-num>15204968</accession-num><label>782883</label><urls><related-urls><url>http://dx.doi.org/10.1080/01926230490465874</url></related-urls></urls><electronic-resource-num>10.1080/01926230490465874</electronic-resource-num><language>English</language></record></Cite></EndNote>], so this could be an artifact of the decreased body weights in the DIBP- and MIBP-treated mice. Nevertheless, results reflect a potential difference in species sensitivity, with rats being more sensitive than mice for effects on testis weight. Overall, reduced testis weight was observed in both species and was consistent with effects observed in rats exposed to DIBP during gestation, so the evidence for effects on male reproductive organ weight was found to be *robust*.

DIBP- or MIBP-exposed rats had increased testicular atrophy (defined as marked or total loss of germinal epithelium of the seminiferous tubules) [ADDIN EN.CITE <EndNote><Cite><Author>Foster</Author><Year>1981</Year><RecNum>77</RecNum><DisplayText>(Foster et al. 1981)</DisplayText><record><rec-number>77</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059055">77</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Foster, P. M.</author><author>Lake, B. G.</author><author>Thomas, L. V.</author><author>Cook, M. W.</author><author>Gangolli, S. D.</author></authors></contributors><titles><title>Testicular effects and zinc excretion produced by various isomers of monobutyl-o-phthalate in the rat</title><secondary-title>Chemico-Biological Interactions</secondary-title><alt-title>Chem Biol Interact</alt-title></titles><periodical><full-title>Chemico-Biological Interactions</full-title><abbr-1>Chem Biol Interact</abbr-1></periodical><alt-periodical><full-title>Chemico-Biological Interactions</full-title><abbr-1>Chem Biol Interact</abbr-1></alt-periodical><pages>233-238</pages><volume>34</volume><number>2</number><dates><year>1981</year></dates><isbn>ISSN 0009-2797
EISSN 1872-7786</isbn><accession-num>7460085</accession-num><label>815876</label><urls><related-urls><url>http://dx.doi.org/10.1016/0009-2797(81)90134-4</url></related-urls></urls><electronic-resource-num>10.1016/0009-2797(81)90134-4</electronic-resource-num><language>English</language></record></Cite></EndNote>] and decreased spermatocytes and spermatogonia compared to controls [ADDIN EN.CITE ADDIN EN.CITE.DATA]. These results are considered *low confidence* because authors provided little information on their methods, and neither study provided quantitative results for the observed effects on sperm. However, results are consistent with those observed in rodents exposed to DIBP during gestation [ADDIN EN.CITE ADDIN EN.CITE.DATA], and are corroborated by the reported decrease in testis weight in these studies. Evidence for testicular degeneration and effects on sperm was therefore found to be *moderate*.

3.4.3. Synthesis of results for male reproductive effects

Overall, the available studies in rodents provide *robust* evidence that DIBP causes male reproductive toxicity (Table 2). Across the gestational exposure studies, male rats had decreased fetal testicular T and displayed the hallmarks of phthalate syndrome (reduced male reproductive organ weights, hypospadias, cryptorchidism, nipple retention, reduced AGD, germ cell effects), and male mice had decreased T and effects on sperm. These effects are consistent with well-established MOAs for male reproductive effects of phthalates, which act through both androgen-dependent (decreased steroidogenesis leading to decreased androgens) and androgen-independent (decreased INSL3 and germ cell effects) pathways [ADDIN EN.CITE <EndNote><Cite><Author>Arzuaga</Author><Year>, in preparation</Year><RecNum>289</RecNum><DisplayText>(Arzuaga et al., in preparation)</DisplayText><record><rec-number>289</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1523359129">289</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Arzuaga, X.</author><author>Walker, T.</author><author>Cooper, G.</author><author>Hotchkiss, A.</author><author>Yost, E.</author></authors></contributors><titles><title>Application of the adverse outcome pathway (AOP) framework to assess species concordance and human relevance of dibutyl phthalate (DBP)-induced male reproductive toxicity </title></titles><dates><year>, in preparation</year></dates><urls></urls></record></Cite></EndNote>], and are supported by mechanistic data in both rats and mice showing decreased fetal testicular expression of steroidogenic genes/proteins and INSL3. While not all studies reported statistical significance, the direction of effect observed for most outcomes was consistent across the available gestational exposure studies, although some biomarkers observed in rats (decreased AGD and decreased adult male reproductive organ weights) were not observed in the mouse study by [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Wang</Author><Year>2017</Year><RecNum>80</RecNum><DisplayText>Wang et al. (2017)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059737">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, X.</author><author>Sheng, N.</author><author>Cui, R.</author><author>Zhang, H.</author><author>Wang, J.</author><author>Dai, J.</author></authors></contributors><titles><title>Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring</title><secondary-title>Chemosphere</secondary-title><alt-title>Chemosphere</alt-title></titles><periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></periodical><alt-periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></alt-periodical><pages>260-267</pages><volume>172</volume><dates><year>2017</year></dates><isbn>ISSN 0045-6535
EISSN 1879-1298</isbn><accession-num>28081510</accession-num><label>3483278</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.chemosphere.2017.01.011</url></related-urls></urls><electronic-resource-num>10.1016/j.chemosphere.2017.01.011</electronic-resource-num><language>English</language></record></Cite></EndNote>].

The database of postnatal exposure studies had more risk of bias and sensitivity concerns compared to the gestational exposure database, but likewise provides evidence of both the androgen-dependent MOA (decreased testis weights) and androgen-independent MOA (increased testicular atrophy and decreased spermatocytes). Although androgens were inconsistently affected across the available postnatal exposure studies for DIBP, it is important to note that pubertal exposure studies for other

antiandrogenic phthalates [e.g. DBP and diethylhexyl phthalate (DEHP)] report decreased T as well as androgen-dependent and -independent effects on reproductive development [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The inconsistent results for DIBP may reflect weaknesses in the design of the available postnatal exposure studies.

Strengths of this database include the availability of several *high confidence* gestational exposure studies, with one large multi-dose study that assessed multiple outcomes in postnatal and adult animals that had been exposed to DIBP in utero during the critical window for male reproductive development [ADDIN EN.CITE <EndNote><Cite><Author>Saillenfait</Author><Year>2008</Year><RecNum>82</RecNum><DisplayTex t>(Saillenfait et al. 2008)</DisplayText><record><rec-number>82</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059943">82</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><pages>107-115</pages><volume>26</volume><number>2</number><dates><year>2008</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>18706996</accession-num><label>680390</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2008.07.006</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2008.07.006</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Other strengths are the availability of studies in both rats and mice that assessed similar outcomes, including multi-dose studies in multiple rat strains. Although none of the studies included a direct measurement of male fertility (e.g. mating success), the observed increase in hypospadias, testicular atrophy, and germ cell effects (including azoospermia) suggest that fertility can be affected.

3.5. Female reproductive effects

Figures indicating the doses at which statistically significant female reproductive effects occurred are provided in the Supplementary Materials (Figures S6 – S7).

3.5.1. Summary of gestational (F1) and postnatal exposure studies

Following gestational exposure, [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Saillenfait</Author><Year>2006</Year><RecNum>8</RecNum><DisplayText> Saillenfait et al. (2006)</DisplayText><record><rec-number>8</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460688">8</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabate, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Developmental toxic effects of diisobutyl phthalate, the methyl-branched analogue of di-n-butyl phthalate, administered by gavage to rats</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-

title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>39-46</pages><volume>165</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>16516415</accession-num><label>680389</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.toxlet.2006.01.013</url></related-urls></urls><electronic-resource-num>10.1016/j.toxlet.2006.01.013</electronic-resource-num><language>English</language></record></Cite></EndNote>] observed a non-significant increase in the incidence of fetuses with displaced ovaries, and [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Borch</Author><Year>2006</Year><RecNum>88</RecNum><DisplayText>Borch et al. (2006)</DisplayText><record><rec-number>88</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060127">88</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Borch, J.</author><author>Axelstad, M.</author><author>Vinggaard, A. M.</author><author>Dalgaard, M.</author></authors></contributors><titles><title>Diisobutyl phthalate has comparable anti-androgenic effects to di-n-butyl phthalate in fetal rat testis</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>183-190</pages><volume>163</volume><number>3</number><dates><year>2006</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>16458459</accession-num><label>674974</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.toxlet.2005.10.020</url></related-urls></urls><electronic-resource-num>10.1016/j.toxlet.2005.10.020</electronic-resource-num><language>English</language></record></Cite></EndNote>] reported a significant increase in female AGD/cubic root of body weight. [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Saillenfait</Author><Year>2008</Year><RecNum>82</RecNum><DisplayText>Saillenfait et al. (2008)</DisplayText><record><rec-number>82</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059943">82</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><pages>107-115</pages><volume>26</volume><number>2</number><dates><year>2008</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>18706996</accession-num><label>680390</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2008.07.006</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2008.07.006</electronic-resource-num><language>English</language></record></Cite></EndNote>] found a slight but not statistically

significant increase in female AGD following gestational exposure, with body weight used as a covariate in their statistical analysis. Although data is limited and the biological implications are unclear, the effects on AGD and ovarian displacement suggest morphological changes in females that are analogous to those observed in male offspring from these studies. Overall, the evidence for effects on female morphological development is considered *slight*.

In female weanling rats exposed to DIBP for up to 20 days, [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Sedha</Author><Year>2015</Year><RecNum>94</RecNum><DisplayText>Sedha et al. (2015)</DisplayText><record><rec-number>94</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060719">94</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sedha, S.</author><author>Gautam, A. K.</author><author>Verma, Y.</author><author>Ahmad, R.</author><author>Kumar, S.</author></authors></contributors><titles><title>Determination of in vivo estrogenic potential of Di-isobutyl phthalate (DIBP) and Di-isononyl phthalate (DINP) in rats</title><secondary-title>Environmental Science and Pollution Research</secondary-title><alt-title>Environ Sci Pollut Res Int</alt-title></titles><periodical><full-title>Environmental Science and Pollution Research</full-title><abbr-1>Environ Sci Pollut Res Int</abbr-1></periodical><alt-periodical><full-title>Environmental Science and Pollution Research</full-title><abbr-1>Environ Sci Pollut Res Int</abbr-1></alt-periodical><pages>18197-18202</pages><volume>22</volume><number>22</number><dates><year>2015</year></dates><isbn>ISSN 0944-1344
EISSN 1614-7499</isbn><accession-num>26178834</accession-num><label>3052887</label><urls><related-urls><url>http://dx.doi.org/10.1007/s11356-015-5021-6</url></related-urls></urls><electronic-resource-num>10.1007/s11356-015-5021-6</electronic-resource-num><language>English</language></record></Cite></EndNote>] found no effect on the timing of female puberty (vaginal opening), and no effects on uterine, paired ovary, or vaginal weight. Because only one study was available, the evidence for effects on morphological development and reproductive organ weight in developing females is considered *indeterminate*.

3.5.2. Summary of maternal (F0) exposure studies

Only one study [ADDIN EN.CITE <EndNote><Cite><Author>Saillenfait</Author><Year>2008</Year><RecNum>82</RecNum><DisplayText>(Saillenfait et al. 2008)</DisplayText><record><rec-number>82</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059943">82</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><pages>107-115</pages><volume>26</volume><number>2</number><dates><year>2008</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>18706996</accession-num><label>680390</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2008.07.006</url></related-urls></urls><electronic-

ED_012964_00000438-00032

resource-num>10.1016/j.reprotox.2008.07.006</electronic-resource-num><language>English</language></record></Cite></EndNote>] evaluated gestation length, and reported that there was no effect of DIBP exposure. The evidence for effects on gestation length was considered *indeterminate*.

Maternal body weight parameters were reported in all gestational exposure studies. Of the studies judged to be *high confidence* for this outcome, [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>BASF</Author><Year>2007</Year><RecNum>81</RecNum><DisplayText>BASF (2007)</DisplayText><record><rec-number>81</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059807">81</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>BASF,</author></authors></contributors><titles><title>Diisobutylphthalate - prenatal developmental toxicity study in Wistar rats administration in the diet (2007 Update)</title></titles><pages>68-155</pages><dates><year>2007</year></dates><publisher>Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d</publisher><isbn>Document Control Number: 86070000046</isbn><label>2259650</label><work-type>TSCA Submission</work-type><urls><related-urls><url>http://yosemite.epa.gov/oppts/epatscat8.nsf/by+Service/82FC6103C0E2F95585257B5100479E79/$File/86070000046.pdf</url></related-urls></urls></record></Cite></EndNote>] reported a significant decrease in maternal body weight gain in Wistar rats after correcting for gravid uterine weight, whereas three other rat studies reported no effect on corrected maternal body weight gain [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The remaining studies were judged to have lower confidence because they did not correct for gravid uterine weight. Of these, [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Howdeshell</Author><Year>2008</Year><RecNum>84</RecNum><DisplayText>Howdeshell et al. (2008)</DisplayText><record><rec-number>84</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060024">84</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Howdeshell, K. L.</author><author>Wilson, V. S.</author><author>Furr, J.</author><author>Lambright, C. R.</author><author>Rider, C. V.</author><author>Blystone, C. R.</author><author>Hotchkiss, A. K.</author><author>Gray, L. E., Jr.</author></authors></contributors><titles><title>A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>153-165</pages><volume>105</volume><number>1</number><dates><year>2008</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>18411233</accession-num><label>675206</label><urls><related-urls><url>http://dx.doi.org/10.1093/toxsci/kfn077</url></related-urls></urls><electronic-resource-num>10.1093/toxsci/kfn077</electronic-resource-num><language>English</language></record></Cite></EndNote>] observed a decrease in maternal body weight gain, which appears to be a consequence of fetal toxicity (decreased fetal body weights); and the remaining studies reported no effect on maternal weight gain [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Overall, the evidence for effects on maternal body weight gain are considered *slight*.

[ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Saillenfait</Author><Year>2006</Year><RecNum>8</RecNum><DisplayText>Saillenfait et al. (2006)</DisplayText><record><rec-number>8</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460688">8</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabate, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Developmental toxic effects of diisobutyl phthalate, the methyl-branched analogue of di-n-butyl phthalate, administered by gavage to rats</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>39-46</pages><volume>165</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>16516415</accession-num><label>680389</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.toxlet.2006.01.013</url></related-urls></urls><electronic-resource-num>10.1016/j.toxlet.2006.01.013</electronic-resource-num><language>English</language></record></Cite></EndNote>] reported a significant reduction in gravid uterine weight, which appears to be a secondary effect to reduced fetal body weights in this study, whereas [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>BASF</Author><Year>2007</Year><RecNum>81</RecNum><DisplayText>BASF (2007)</DisplayText><record><rec-number>81</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059807">81</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>BASF,</author></authors></contributors><titles><title>Diisobutylphthalate - prenatal developmental toxicity study in Wistar rats administration in the diet (2007 Update)</title></titles><pages>68-155</pages><dates><year>2007</year></dates><publisher>Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d</publisher><isbn>Document Control Number: 86070000046</isbn><label>2259650</label><work-type>TSCA Submission</work-type><urls><related-urls><url>http://yosemite.epa.gov/oppts/epatscat8.nsf/by+Service/82FC6103C0E2F95585257B5100479E79/$File/86070000046.pdf</url></related-urls></urls></record></Cite></EndNote>] and [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Saillenfait</Author><Year>2017</Year><RecNum>83</RecNum><DisplayText>Saillenfait et al. (2017)</DisplayText><record><rec-number>83</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059944">83</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Denis, F.</author><author>Antoine, G.</author><author>Robert, A.</author><author>Roudot, A. C.</author><author>Ndiaye, D.</author><author>Eljarrat, E.</author></authors></contributors><titles><title>Evaluation of the effects of α-cypermethrin on fetal rat testicular steroidogenesis</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-

title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><dates><year>2017</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>28655647</accession-num><label>3859062</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2017.06.133</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2017.06.133</electronic-resource-num><language>English</language></record></Cite></EndNote>] reported no effect on gravid uterine weight. Given this limited amount of information, the evidence for effects on maternal organ weights is considered *indeterminate*.

3.5.3. Synthesis of results for female reproductive effects:

Overall, the available studies provide *slight* evidence that DIBP causes female reproductive toxicity following developmental or maternal exposure (Table 3). This database has significant limitations. The exposure durations used in the gestational exposure studies were selected primarily for evaluating male reproductive toxicity and fetal morphological development, and may not be the most sensitive for detecting maternal toxicity. None of the studies included pre-mating, mating, or lactational exposure intervals; therefore, effects on fertility, mating, fecundity (other than litter size effects due to post-implantation loss), and estrous cyclicity were not evaluated. Additionally, limited data were presented on effects in F1 females exposed during gestation, compared to the detailed evaluations of reproductive toxicity in F1 males.

3.6. Developmental effects

Figures indicating the doses at which statistically significant developmental effects occurred are provided in the Supplementary Materials (Figures S8 – S10).

3.6.1. Summary of gestational (F1) exposure studies

Two *high confidence* studies [ADDIN EN.CITE ADDIN EN.CITE.DATA] that exposed Sprague-Dawley rats by gavage beginning soon after the time of implantation (GD 6-20 and GD 8-18, respectively) observed a dose-related increase in fetal resorptions per litter, leading to a 52-62% decrease in the number of live fetuses per litter. In contrast, effects on fetal viability were not observed in the other studies that reported this outcome, including two dietary studies that exposed Wistar rats for similar durations [ADDIN EN.CITE ADDIN EN.CITE.DATA], four studies that exposed Sprague-Dawley rats for shorter durations in mid- to late gestation [ADDIN EN.CITE ADDIN EN.CITE.DATA], or in mice exposed throughout gestation [ADDIN EN.CITE <EndNote><Cite><Author>Wang</Author><Year>2017</Year><RecNum>80</RecNum><DisplayText>(Wang et al. 2017)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059737">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, X.</author><author>Sheng, N.</author><author>Cui, R.</author>Zhang, H.</author><author>Wang, J.</author><author>Dai, J.</author></authors></contributors><titles><title>Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring</title><secondary-title>Chemosphere</secondary-title><alt-title>Chemosphere</alt-title></titles><periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></periodical><alt-periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></alt-periodical><pages>260-

267</pages><volume>172</volume><dates><year>2017</year></dates><isbn>ISSN 0045-6535
EISSN 1879-1298</isbn><accession-num>28081510</accession-num><label>3483278</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.chemosphere.2017.01.011</url></related-urls></urls><electronic-resource-num>10.1016/j.chemosphere.2017.01.011</electronic-resource-num><language>English</language></record></Cite></EndNote>]. This variability in results appears to be driven by differences in species, strain, and study design (particularly exposure duration). Reduced fetal survival have also been observed for other phthalates, such as DBP, and evidence suggests that DBP can interfere with pregnancy maintenance by decreasing ovarian progesterone production [ADDIN EN.CITE <EndNote><Cite><Author>Gray</Author><Year>2006</Year><RecNum>7</RecNum><DisplayText>(Gray et al. 2006)</DisplayText><record><rec-number>7</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460657">7</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gray, L. E., Jr.</author><author>Laskey, J.</author><author>Ostby, J.</author></authors></contributors><titles><title>Chronic di-n-butyl phthalate exposure in rats reduces fertility and alters ovarian function during pregnancy in female Long Evans hooded rats</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>189-195</pages><volume>93</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>16763070</accession-num><label>673276</label><urls><related-urls><url>http://dx.doi.org/10.1093/toxsci/kfl035</url></related-urls></urls><electronic-resource-num>10.1093/toxsci/kfl035</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Given this biologically plausible mechanistic hypothesis for other phthalates, and the large effect size and exposure-response gradient observed in [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Howdeshell</Author><Year>2008</Year><RecNum>84</RecNum><DisplayText>Howdeshell et al. (2008)</DisplayText><record><rec-number>84</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060024">84</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Howdeshell, K. L.</author><author>Wilson, V. S.</author><author>Furr, J.</author><author>Lambright, C. R.</author><author>Rider, C. V.</author><author>Blystone, C. R.</author><author>Hotchkiss, A. K.</author><author>Gray, L. E., Jr.</author></authors></contributors><titles><title>A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>153-165</pages><volume>105</volume><number>1</number><dates><year>2008</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>18411233</accession-

num><label>675206</label><urls><related-urls><url>http://dx.doi.org/10.1093/toxsci/kfn077</url></related-urls></urls><electronic-resource-num>10.1093/toxsci/kfn077</electronic-resource-num><language>English</language></record></Cite></EndNote>] and [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Saillenfait</Author><Year>2006</Year><RecNum>8</RecNum><DisplayText>Saillenfait et al. (2006)</DisplayText><record><rec-number>8</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460688">8</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabate, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Developmental toxic effects of diisobutyl phthalate, the methyl-branched analogue of di-n-butyl phthalate, administered by gavage to rats</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>39-46</pages><volume>165</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>16516415</accession-num><label>680389</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.toxlet.2006.01.013</url></related-urls></urls><electronic-resource-num>10.1016/j.toxlet.2006.01.013</electronic-resource-num><language>English</language></record></Cite></EndNote>], the evidence for effects on fetal survival was found to be *robust*.

Postnatal survival following gestational exposure was only evaluated by [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Saillenfait</Author><Year>2006</Year><RecNum>8</RecNum><DisplayText>Saillenfait et al. (2006)</DisplayText><record><rec-number>8</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460688">8</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabate, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Developmental toxic effects of diisobutyl phthalate, the methyl-branched analogue of di-n-butyl phthalate, administered by gavage to rats</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>39-46</pages><volume>165</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>16516415</accession-num><label>680389</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.toxlet.2006.01.013</url></related-urls></urls><electronic-resource-num>10.1016/j.toxlet.2006.01.013</electronic-resource-num><language>English</language></record></Cite></EndNote>], who found no effect on the survival of male and female rat pups through weaning. The evidence for effects on postnatal survival was found to be *indeterminate*.

Most studies in rats reported a dose-related decrease in fetal body weights [ADDIN EN.CITE ADDIN EN.CITE.DATA] and postnatal and adult body weights [ADDIN EN.CITE <EndNote><Cite><Author>Saillenfait</Author><Year>2008</Year><RecNum>82</RecNum><DisplayText>(Saillenfait et al. 2008)</DisplayText><record><rec-number>82</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059943">82</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><pages>107-115</pages><volume>26</volume><number>2</number><dates><year>2008</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>18706996</accession-num><label>680390</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2008.07.006</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2008.07.006</electronic-resource-num><language>English</language></record></Cite></EndNote>] of animals exposed to DIBP during gestation. No effects on body weight were observed in rats by [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Saillenfait</Author><Year>2017</Year><RecNum>83</RecNum><DisplayText>Saillenfait et al. (2017)</DisplayText><record><rec-number>83</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059944">83</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Denis, F.</author><author>Antoine, G.</author><author>Robert, A.</author><author>Roudot, A. C.</author><author>Ndiaye, D.</author><author>Eljarrat, E.</author></authors></contributors><titles><title>Evaluation of the effects of α-cypermethrin on fetal rat testicular steroidogenesis</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><dates><year>2017</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>28655647</accession-num><label>3859062</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2017.06.133</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2017.06.133</electronic-resource-num><language>English</language></record></Cite></EndNote>] or in mice by [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Wang</Author><Year>2017</Year><RecNum>80</RecNum><DisplayText>Wang et al. (2017)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059737">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, X.</author><author>Sheng, N.</author><author>Cui, R.</author><author>Zhang, H.</author><author>Wang, J.</author><author>Dai,

J.</author></authors></contributors><titles><title>Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring</title><secondary-title>Chemosphere</secondary-title><alt-title>Chemosphere</alt-title></titles><periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></periodical><alt-periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></alt-periodical><pages>260-267</pages><volume>172</volume><dates><year>2017</year></dates><isbn>ISSN 0045-6535
EISSN 1879-1298</isbn><accession-num>28081510</accession-num><label>3483278</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.chemosphere.2017.01.011</url></related-urls></urls><electronic-resource-num>10.1016/j.chemosphere.2017.01.011</electronic-resource-num><language>English</language></record></Cite></EndNote>], although these two studies tested a single lower dose of DIBP. Given the exposure-response gradient observed in studies that tested a wider range of DIBP doses, the evidence for effects on prenatal growth was found to be *robust*, and evidence for effects on postnatal growth after gestational exposure was found to be *moderate*.

Different results were observed in the two studies that performed detailed analyses of fetal external, skeletal, and soft tissue/visceral malformations and variations following exposure from GD 6-20. In Sprague-Dawley rats, [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Saillenfait</Author><Year>2006</Year><RecNum>8</RecNum><DisplayText>Saillenfait et al. (2006)</DisplayText><record><rec-number>8</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460688">8</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabate, J. P.</author><author>Gallissot, F.</author></authors></contributors><titles><title>Developmental toxic effects of diisobutyl phthalate, the methyl-branched analogue of di-n-butyl phthalate, administered by gavage to rats</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>39-46</pages><volume>165</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>16516415</accession-num><label>680389</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.toxlet.2006.01.013</url></related-urls></urls><electronic-resource-num>10.1016/j.toxlet.2006.01.013</electronic-resource-num><language>English</language></record></Cite></EndNote>] reported a dose-related increase in the total incidence of external, skeletal, and visceral malformations in fetuses following gestational exposure to DIBP, reaching statistical significance at 750 and 1000 mg/kg-day. In particular, the incidence of fused sternebrae increased statistically significantly with dose, and a variety of other individual malformations of the neural tube, eye, vessels of the heart, vertebral column, and axial skeleton exhibited dose-related increases that were not significant. Dose-related skeletal variations included supernumerary ribs, incomplete ossification of thoracic or lumbar vertebral centra, and assorted variations of the skull and axial skeleton. Dose-related visceral variations included dilation or distention of the ureter and dilated renal pelvis, as well as ectopic testis and displaced ovaries (discussed in the male and female reproductive sections, respectively). Comparatively, in Wistar rats, [ADDIN EN.CITE <EndNote><Cite

AuthorYear="1"><Author>BASF</Author><Year>2007</Year><RecNum>81</RecNum><DisplayText>BASF (2007)</DisplayText><record><rec-number>81</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059807">81</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>BASF,</author></authors></contributors><titles><title>Diisobutylphthalate - prenatal developmental toxicity study in Wistar rats administration in the diet (2007 Update)</title></titles><pages>68-155</pages><dates><year>2007</year></dates><publisher>Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d</publisher><isbn>Document Control Number: 86070000046</isbn><label>2259650</label><work-type>TSCA Submission</work-type><urls><related-urls><url>http://yosemite.epa.gov/oppts/epatscat8.nsf/by+Service/82FC6103C0E2F95585257B5100479E79/$File/86070000046.pdf</url></related-urls></urls></record></Cite></EndNote>] did not observe any dose-related trends at doses up to 942 mg/kg-day. In some cases, the frequency of skeletal variations was significantly elevated in treatment groups relative to control; however, the incidences of these variations were consistently within the range of historical control data for that laboratory, suggesting that they were not caused by DIBP treatment. Additionally, no effects on external morphology were observed by [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Saillenfait</Author><Year>2017</Year><RecNum>83</RecNum><DisplayText>Saillenfait et al. (2017)</DisplayText><record><rec-number>83</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059944">83</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Saillenfait, A. M.</author><author>Sabaté, J. P.</author><author>Denis, F.</author><author>Antoine, G.</author><author>Robert, A.</author><author>Roudot, A. C.</author><author>Ndiaye, D.</author><author>Eljarrat, E.</author></authors></contributors><titles><title>Evaluation of the effects of α-cypermethrin on fetal rat testicular steroidogenesis</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title></titles><periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></periodical><alt-periodical><full-title>Reproductive Toxicology</full-title><abbr-1>Reprod Toxicol</abbr-1></alt-periodical><dates><year>2017</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>28655647</accession-num><label>3859062</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.reprotox.2017.06.133</url></related-urls></urls><electronic-resource-num>10.1016/j.reprotox.2017.06.133</electronic-resource-num><language>English</language></record></Cite></EndNote>] at the single dose tested (250 mg/kg-day). Given that a clear exposure-response gradient was observed in one rat study while no effects were observed in the others, the evidence for effects on fetal morphological development was found to be *slight*.

3.6.2. Summary of postnatal exposure studies

A dose-related decrease in postnatal and adult growth was observed in all available studies that exposed immature rats and mice [ADDIN EN.CITE ADDIN EN.CITE.DATA]. However, these studies were judged to be *medium* or *low confidence* for this outcome because of their reporting limitations; and, in the studies by Oishi and Hiraga, decreased growth may have been a secondary effect related to decreased

food consumption in the DIBP or MIBP treatment groups. Overall, the evidence for effects on postnatal growth was found to be *moderate.*

3.6.3. Synthesis of results for developmental effects

Overall, the available studies in rodents provide *robust* evidence that DIBP causes developmental toxicity (Table 4). The strongest evidence was available from the gestational exposure studies, which found effects on survival, growth, and fetal structural abnormalities. One limitation is that many of the studies exposed fetuses only during the major window of male sexual differentiation (e.g. GD 14-18), which may not be the most sensitive for detecting effects on survival or on skeleton or organ system development, and there were no two-generation or continuous breeding studies available. This limits the ability to fully evaluate potential effects on the developing fetus; for instance, none of the rat studies exposed dams prior to implantation, so it was not possible to evaluate effects on preimplantation loss. Nevertheless, the targeted dosing periods are informative of developmental hazards overall, and may provide a starting point for understanding the windows of susceptibility for various outcomes in the developing fetus.

3.7. Liver effects

A figure indicating the doses at which statistically significant effects on liver weight occurred is provided in the Supplementary Materials (Figure S11).

3.7.1. Summary of available studies

Dose-related increases in relative liver weight were observed in all studies that reported this outcome, consisting of postnatal exposure studies in male rats [ADDIN EN.CITE ADDIN EN.CITE.DATA], female rats [ADDIN EN.CITE <EndNote><Cite><Author>University of Rochester</Author><Year>1954</Year><RecNum>72</RecNum><DisplayText>(University of Rochester 1954)</DisplayText><record><rec-number>72</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058725">72</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>University of Rochester,</author></authors></contributors><titles><title>Preliminary acute toxicity tests and short term feeding tests of rats and dogs given di-isobutyl phthalate and di-butyl phthalate</title></titles><dates><year>1954</year></dates><pub-location>Rochester, NY</pub-location><publisher>University of Rochester School of Medicine and Dentistry</publisher><isbn>EPA/OTS Doc #878210833</isbn><label>680305</label><work-type>TSCA Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0205995</url></related-urls></urls><language>English</language></record></Cite></EndNote>], and male mice [ADDIN EN.CITE ADDIN EN.CITE.DATA]; and in adult male mice that were exposed during gestation [ADDIN EN.CITE <EndNote><Cite><Author>Wang</Author><Year>2017</Year><RecNum>80</RecNum><DisplayText>(Wang et al. 2017)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059737">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, X.</author><author>Sheng, N.</author><author>Cui, R.</author><author>Zhang, H.</author><author>Wang, J.</author><author>Dai,

J.</author></authors></contributors><titles><title>Gestational and lactational exposure to di-isobutyl phthalate via diet in maternal mice decreases testosterone levels in male offspring</title><secondary-title>Chemosphere</secondary-title><alt-title>Chemosphere</alt-title></titles><periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></periodical><alt-periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1></alt-periodical><pages>260-267</pages><volume>172</volume><dates><year>2017</year></dates><isbn>ISSN 0045-6535
EISSN 1879-1298</isbn><accession-num>28081510</accession-num><label>3483278</label><urls><related-urls><url>http://dx.doi.org/10.1016/j.chemosphere.2017.01.011</url></related-urls></urls><electronic-resource-num>10.1016/j.chemosphere.2017.01.011</electronic-resource-num><language>English</language></record></Cite></EndNote>]. While the University of Rochester studies did not include a statistical analysis, the average magnitude of effect was large (increased by up to 84% compared to controls); and in all other cases, effects on relative liver weight were statistically significant. Statistically significant increases in absolute liver weight were also observed in some cases [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The largest increases in liver weight were often seen at doses associated with marked growth retardation, which has the potential to exaggerate the effect of relative weight measurements; however, since the relationship between liver weight and body weight is proportional, changes in relative liver weight are considered informative of toxicity [ADDIN EN.CITE <EndNote><Cite><Author>Bailey</Author><Year>2004</Year><RecNum>102</RecNum><DisplayText>(Bailey et al. 2004)</DisplayText><record><rec-number>102</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519066625">102</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bailey, S. A.</author><author>Zidell, R. H.</author><author>Perry, R. W.</author></authors></contributors><titles><title>Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint?</title><secondary-title>Toxicologic Pathology</secondary-title><alt-title>Toxicol Pathol</alt-title></titles><periodical><full-title>Toxicologic Pathology</full-title><abbr-1>Toxicol Pathol</abbr-1></periodical><alt-periodical><full-title>Toxicologic Pathology</full-title><abbr-1>Toxicol Pathol</abbr-1></alt-periodical><pages>448-466</pages><volume>32</volume><number>4</number><dates><year>2004</year></dates><isbn>ISSN 0192-6233
EISSN 1533-1601</isbn><accession-num>15204968</accession-num><label>782883</label><urls><related-urls><url>http://dx.doi.org/10.1080/01926230490465874</url></related-urls></urls><electronic-resource-num>10.1080/01926230490465874</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Given the consistent direction of effect across studies and two species, the evidence for effects on liver weight is considered *robust*.

Two of these studies also included a histopathological analysis [ADDIN EN.CITE <EndNote><Cite><Author>University of Rochester</Author><Year>1953</Year><RecNum>71</RecNum><DisplayText>(University of Rochester 1953, 1954)</DisplayText><record><rec-number>71</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058725">71</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>University of Rochester,</author></authors></contributors><titles><title>One month feeding tests of di-isobutyl phthalate with cover letter</title></titles><dates><year>1953</year></dates><pub-

location>Rochester, NY</pub-location><publisher>University of Rochester School of Medicine and Dentistry</publisher><isbn>EPA/OTS Doc #878212229</isbn><label>680304</label><work-type>TSCA Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0205995</url></related-urls></urls><language>English</language></record></Cite><Cite><Author>University of Rochester</Author><Year>1954</Year><RecNum>72</RecNum><record><rec-number>72</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058725">72</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>University of Rochester,</author></authors></contributors><titles><title>Preliminary acute toxicity tests and short term feeding tests of rats and dogs given di-isobutyl phthalate and di-butyl phthalate</title></titles><dates><year>1954</year></dates><pub-location>Rochester, NY</pub-location><publisher>University of Rochester School of Medicine and Dentistry</publisher><isbn>EPA/OTS Doc #878210833</isbn><label>680305</label><work-type>TSCA Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0205995</url></related-urls></urls><language>English</language></record></Cite></EndNote>], and found no difference between treatment and control livers. However, these results are considered *low confidence* because of reporting limitations in these studies. Therefore, the evidence for histopathological effects in the liver is considered *indeterminate*.

3.7.2. Synthesis of results for liver effects

Change in organ weight alone has been used as a potentially sensitive indicator for toxicity from chemical exposure [ADDIN EN.CITE <EndNote><Cite><Author>Bailey</Author><Year>2004</Year><RecNum>102</RecNum><DisplayText>(Bailey et al. 2004)</DisplayText><record><rec-number>102</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519066625">102</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bailey, S. A.</author><author>Zidell, R. H.</author><author>Perry, R. W.</author></authors></contributors><titles><title>Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint?</title><secondary-title>Toxicologic Pathology</secondary-title><alt-title>Toxicol Pathol</alt-title></titles><periodical><full-title>Toxicologic Pathology</full-title><abbr-1>Toxicol Pathol</abbr-1></periodical><alt-periodical><full-title>Toxicologic Pathology</full-title><abbr-1>Toxicol Pathol</abbr-1></alt-periodical><pages>448-466</pages><volume>32</volume><number>4</number><dates><year>2004</year></dates><isbn>ISSN 0192-6233
EISSN 1533-1601</isbn><accession-num>15204968</accession-num><label>782883</label><urls><related-urls><url>http://dx.doi.org/10.1080/01926230490465874</url></related-urls></urls><electronic-resource-num>10.1080/01926230490465874</electronic-resource-num><language>English</language></record></Cite></EndNote>]. In the absence of histopathological changes or supporting mechanistic evidence (e.g. changes in hepatic enzyme expression), however, the biological significance of the reported changes in liver weight is inconclusive [ADDIN EN.CITE <EndNote><Cite><Author>Hall</Author><Year>2012</Year><RecNum>103</RecNum><DisplayText>(H

all et al. 2012)</DisplayText><record><rec-number>103</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519066803">103</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hall, A. P.</author><author>Elcombe, C. R.</author><author>Foster, J. R.</author><author>Harada, T.</author><author>Kaufmann, W.</author><author>Knippel, A.</author><author>Küttler, K.</author><author>Malarkey, D. E.</author><author>Maronpot, R. R.</author><author>Nishikawa, A.</author><author>Nolte, T.</author><author>Schulte, A.</author><author>Strauss, V.</author><author>York, M. J.</author></authors></contributors><titles><title>Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop</title><secondary-title>Toxicologic Pathology</secondary-title><alt-title>Toxicol Pathol</alt-title></titles><periodical><full-title>Toxicologic Pathology</full-title><abbr-1>Toxicol Pathol</abbr-1></periodical><alt-periodical><full-title>Toxicologic Pathology</full-title><abbr-1>Toxicol Pathol</abbr-1></alt-periodical><pages>971-994</pages><volume>40</volume><number>7</number><dates><year>2012</year></dates><isbn>ISSN 0192-6233
EISSN 1533-1601</isbn><accession-num>22723046</accession-num><label>2718645</label><work-type>Review</work-type><urls><related-urls><url>http://dx.doi.org/10.1177/0192623312448935</url></related-urls></urls><electronic-resource-num>10.1177/0192623312448935</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Taken together, the evidence for liver toxicity is considered *indeterminate* (Table S1).

3.8. ***Kidney effects***

A figure indicating the doses at which statistically significant effects on kidney weight occurred is provided in the Supplementary Materials (Figure S12).

3.8.1. Summary of available studies

Dose-related increases in relatively kidney weight were reported in the 1- and 4-month postnatal exposure studies in male and female rats by the University of Rochester [ADDIN EN.CITE <EndNote><Cite ExcludeAuth="1"><Author>University of Rochester</Author><Year>1953</Year><RecNum>71</RecNum><DisplayText>(1953, 1954)</DisplayText><record><rec-number>71</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058725">71</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>University of Rochester,</author></authors></contributors><titles><title>One month feeding tests of di-isobutyl phthalate with cover letter</title></titles><dates><year>1953</year></dates><pub-location>Rochester, NY</pub-location><publisher>University of Rochester School of Medicine and Dentistry</publisher><isbn>EPA/OTS Doc #878212229</isbn><label>680304</label><work-type>TSCA Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0205995</url></related-urls></urls><language>English</language></record></Cite><Cite ExcludeAuth="1"><Author>University of Rochester</Author><Year>1954</Year><RecNum>72</RecNum><record><rec-number>72</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058725">72</key></foreign-keys><ref-type name="Report">27</ref-

type><contributors><authors><author>University of Rochester,</author></authors></contributors><titles><title>Preliminary acute toxicity tests and short term feeding tests of rats and dogs given di-isobutyl phthalate and di-butyl phthalate</title></titles><dates><year>1954</year></dates><pub-location>Rochester, NY</pub-location><publisher>University of Rochester School of Medicine and Dentistry</publisher><isbn>EPA/OTS Doc #878210833</isbn><label>680305</label><work-type>TSCA Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0205995</url></related-urls></urls><language>English</language></record></Cite></EndNote>], with more pronounced changes in males compared to females [ADDIN EN.CITE <EndNote><Cite><Author>University of Rochester</Author><Year>1954</Year><RecNum>72</RecNum><DisplayText>(University of Rochester 1954)</DisplayText><record><rec-number>72</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058725">72</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>University of Rochester,</author></authors></contributors><titles><title>Preliminary acute toxicity tests and short term feeding tests of rats and dogs given di-isobutyl phthalate and di-butyl phthalate</title></titles><dates><year>1954</year></dates><pub-location>Rochester, NY</pub-location><publisher>University of Rochester School of Medicine and Dentistry</publisher><isbn>EPA/OTS Doc #878210833</isbn><label>680305</label><work-type>TSCA Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0205995</url></related-urls></urls><language>English</language></record></Cite></EndNote>], although the authors did not perform a statistical analysis. In contrast, the 7-day exposure studies found that relative kidney weight was statistically significantly decreased in mice exposed to DIBP [ADDIN EN.CITE <EndNote><Cite><Author>Oishi</Author><Year>1980</Year><RecNum>91</RecNum><DisplayText>(Oishi and Hiraga 1980c)</DisplayText><record><rec-number>91</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060513">91</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Oishi, S.</author><author>Hiraga, K.</author></authors></contributors><titles><title>Effect of phthalic acid esters on mouse testes</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>413-416</pages><volume>5</volume><number>6</number><dates><year>1980</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>7394838</accession-num><label>680366</label><urls><related-urls><url>http://dx.doi.org/10.1016/0378-4274(80)90024-7</url></related-urls></urls><electronic-resource-num>10.1016/0378-4274(80)90024-7</electronic-resource-num><language>English</language></record></Cite></EndNote>], and not significantly changed in mice exposed to MIBP [ADDIN EN.CITE <EndNote><Cite><Author>Oishi</Author><Year>1980</Year><RecNum>90</RecNum><DisplayText>(Oishi and Hiraga 1980b)</DisplayText><record><rec-number>90</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps"

timestamp="1519060475">90</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Oishi, S.</author><author>Hiraga, K.</author></authors></contributors><titles><title>Effects of phthalic acid monoesters on mouse testes</title><secondary-title>Toxicology Letters</secondary-title><alt-title>Toxicol Lett</alt-title></titles><periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></periodical><alt-periodical><full-title>Toxicology Letters</full-title><abbr-1>Toxicol Lett</abbr-1></alt-periodical><pages>239-242</pages><volume>6</volume><number>4-5</number><dates><year>1980</year></dates><isbn>ISSN 0378-4274
EISSN 1879-3169</isbn><accession-num>7423552</accession-num><label>675362</label><urls><related-urls><url>http://dx.doi.org/10.1016/0378-4274(80)90126-5</url></related-urls></urls><electronic-resource-num>10.1016/0378-4274(80)90126-5</electronic-resource-num><language>English</language></record></Cite></EndNote>] or rats exposed to DIBP [ADDIN EN.CITE <EndNote><Cite><Author>Oishi</Author><Year>1980</Year><RecNum>92</RecNum><DisplayText>(Oishi and Hiraga 1980d)</DisplayText><record><rec-number>92</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060555">92</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Oishi, S.</author><author>Hiraga, K.</author></authors></contributors><titles><title>Testicular atrophy induced by phthalic acid esters: Effect on testosterone and zinc concentrations</title><secondary-title>Toxicology and Applied Pharmacology</secondary-title><alt-title>Toxicol Appl Pharmacol</alt-title></titles><periodical><full-title>Toxicology and Applied Pharmacology</full-title><abbr-1>Toxicol Appl Pharmacol</abbr-1></periodical><alt-periodical><full-title>Toxicology and Applied Pharmacology</full-title><abbr-1>Toxicol Appl Pharmacol</abbr-1></alt-periodical><pages>35-41</pages><volume>53</volume><number>1</number><dates><year>1980</year></dates><isbn>ISSN 0041-008X
EISSN 1096-0333</isbn><accession-num>7385236</accession-num><label>61572</label><urls><related-urls><url>http://dx.doi.org/10.1016/0041-008X(80)90378-6</url></related-urls></urls><electronic-resource-num>10.1016/0041-008X(80)90378-6</electronic-resource-num><language>English</language></record></Cite></EndNote>]. In a 4-day exposure of rats to MIBP, relative kidney weight was increased by 12% compared to controls, although the effect was not significant [ADDIN EN.CITE <EndNote><Cite><Author>Foster</Author><Year>1982</Year><RecNum>76</RecNum><DisplayText>(Foster et al. 1982)</DisplayText><record><rec-number>76</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519059054">76</key></foreign-keys><ref-type name="Edited Book">28</ref-type><contributors><authors><author>Foster, P. M. D.</author><author>Lake, B. G.</author><author>Cook, M. W.</author><author>Thomas, L. V.</author><author>Gangolli, S. D.</author></authors><secondary-authors><author>Snyder, R.</author><author>Jollow, D. J.</author><author>Parke, D. V.</author><author>Gibson, C. G.</author><author>Kocsis, J. J.</author><author>Witmer, C. M.</author></secondary-authors></contributors><titles><title>Structure-activity requirements for the induction of testicular atrophy by butyl phthalates in immature rats: Effect on testicular zinc content</title><secondary-title>Advances in Experimental Medicine and Biology</secondary-title></titles><pages>445-452</pages><volume>136</volume><dates><year>1982</year><pub-dates><date>July 14-17, 1980</date></pub-dates></dates><pub-location>New York, NY</pub-location><publisher>Plenum

Press</publisher><accession-num>7344475</accession-num><label>61558</label><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/7344475</url></related-urls></urls><language>English</language></record></Cite></EndNote>]. Given these inconsistencies and the limited number of studies, the overall evidence for effects on kidney weight is considered *slight*.

Two of these studies also included a histopathological analysis [ADDIN EN.CITE <EndNote><Cite><Author>University of Rochester</Author><Year>1953</Year><RecNum>71</RecNum><DisplayText>(University of Rochester 1953, 1954)</DisplayText><record><rec-number>71</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058725">71</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>University of Rochester,</author></authors></contributors><titles><title>One month feeding tests of di-isobutyl phthalate with cover letter</title></titles><dates><year>1953</year></dates><pub-location>Rochester, NY</pub-location><publisher>University of Rochester School of Medicine and Dentistry</publisher><isbn>EPA/OTS Doc #878212229</isbn><label>680304</label><work-type>TSCA Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0205995</url></related-urls></urls><language>English</language></record></Cite><Cite><Author>University of Rochester</Author><Year>1954</Year><RecNum>72</RecNum><record><rec-number>72</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058725">72</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>University of Rochester,</author></authors></contributors><titles><title>Preliminary acute toxicity tests and short term feeding tests of rats and dogs given di-isobutyl phthalate and di-butyl phthalate</title></titles><dates><year>1954</year></dates><pub-location>Rochester, NY</pub-location><publisher>University of Rochester School of Medicine and Dentistry</publisher><isbn>EPA/OTS Doc #878210833</isbn><label>680305</label><work-type>TSCA Submission</work-type><urls><related-urls><url>https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0205995</url></related-urls></urls><language>English</language></record></Cite></EndNote>], and found no difference between treatment and control livers. These histopathology results are considered *low confidence* because of reporting limitations in these studies.

3.8.2. Synthesis of results for kidney effects

Taken together, the available evidence is inadequate to draw conclusions on the effects of DIBP on the kidney. Therefore, the evidence for kidney toxicity is considered *indeterminate* (Table S2).

3.9. *Cancer*

None of the available studies evaluated cancer in animals exposed to DIBP. The limited number of mutagenicity assays identified in the literature search generally had negative findings [ADDIN EN.CITE ADDIN EN.CITE.DATA], although genotoxicity assays of DIBP-treated primary human mucosal cells demonstrated DNA damage as measured by the comet assay [ADDIN EN.CITE ADDIN EN.CITE.DATA]. This was considered inadequate information to evaluate carcinogenicity; thus, the evidence for cancer is considered *indeterminate*.

DISCUSSION:

The results of this systematic review provide *robust* evidence that DIBP causes male reproductive and developmental toxicity and *slight* evidence for female reproductive toxicity, whereas evidence for liver, kidney, and cancer was *indeterminate*. These results corroborate the [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>CHAP</Author><Year>2014</Year><RecNum>4</RecNum><DisplayText>CHAP (2014)</DisplayText><record><rec-number>4</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460456">4</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>CHAP,</author></authors></contributors><titles><title>Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with appendices)</title></titles><dates><year>2014</year></dates><pub-location>Bethesda, MD</pub-location><publisher>U.S. Consumer Product Safety Commission, Directorate for Health Sciences</publisher><label>2439960</label><urls><related-urls><url>http://www.cpsc.gov/en/Regulations-Laws--Standards/Statutes/The-Consumer-Product-Safety-Improvement-Act/Phthalates/Chronic-Hazard-Advisory-Panel-CHAP-on-Phthalates/</url></related-urls></urls></record></Cite></EndNote>] and [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>NAS</Author><Year>2017</Year><RecNum>47</RecNum><DisplayText>NAS (2017)</DisplayText><record><rec-number>47</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509470742">47</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>NAS,</author></authors></contributors><titles><title>Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals</title><secondary-title>Consensus Study Report</secondary-title></titles><dates><year>2017</year></dates><pub-location>Washington, D.C.</pub-location><publisher>The National Academies Press</publisher><label>3982546</label><urls><related-urls><url>http://dx.doi.org/10.17226/24758</url></related-urls></urls><electronic-resource-num>10.17226/24758</electronic-resource-num><language>English</language></record></Cite></EndNote>] systematic review conclusions that DIBP is a male reproductive toxic agent, with gestational exposure leading to permanent adverse effects in male offspring, and support DIBP as a children's health concern. The results also provide a reconnaissance of additional effects observed in laboratory animals after DIBP exposure, some of which may share the same mechanisms as the male reproductive effects.

[ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Howdeshell</Author><Year>2008</Year><RecNum>84</RecNum><DisplayText>Howdeshell et al. (2008)</DisplayText><record><rec-number>84</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519060024">84</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Howdeshell, K. L.</author><author>Wilson, V. S.</author><author>Furr, J.</author><author>Lambright, C. R.</author><author>Rider, C. V.</author><author>Blystone, C. R.</author><author>Hotchkiss, A. K.</author><author>Gray, L. E., Jr.</author></authors></contributors><titles><title>A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive

manner</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>153-165</pages><volume>105</volume><number>1</number><dates><year>2008</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>18411233</accession-num><label>675206</label><urls><related-urls><url>http://dx.doi.org/10.1093/toxsci/kfn077</url></related-urls></urls><electronic-resource-num>10.1093/toxsci/kfn077</electronic-resource-num><language>English</language></record></Cite></EndNote>] demonstrated that fetal testosterone production and fetal mortality (post-implantation loss) followed a similar dose-response relationship in rats exposed to a mixture of phthalates during gestation, and suggested that this might be because fetal T production and fetal mortality are both caused by decreased steroidogenesis (decreased testicular testosterone in male fetuses, and decreased ovarian progesterone in dams). While it has been found that DBP decreases maternal ovarian progesterone production in dams exposed at mid-pregnancy [ADDIN EN.CITE <EndNote><Cite><Author>Gray</Author><Year>2006</Year><RecNum>7</RecNum><DisplayText>(Gray et al. 2006)</DisplayText><record><rec-number>7</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460657">7</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gray, L. E., Jr.</author><author>Laskey, J.</author><author>Ostby, J.</author></authors></contributors><titles><title>Chronic di-n-butyl phthalate exposure in rats reduces fertility and alters ovarian function during pregnancy in female Long Evans hooded rats</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>189-195</pages><volume>93</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>16763070</accession-num><label>673276</label><urls><related-urls><url>http://dx.doi.org/10.1093/toxsci/kfl035</url></related-urls></urls><electronic-resource-num>10.1093/toxsci/kfl035</electronic-resource-num><language>English</language></record></Cite></EndNote>], this has not yet been evaluated for DIBP. [ADDIN EN.CITE <EndNote><Cite AuthorYear="1"><Author>Gray</Author><Year>2006</Year><RecNum>7</RecNum><DisplayText>Gray et al. (2006)</DisplayText><record><rec-number>7</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1509460657">7</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gray, L. E., Jr.</author><author>Laskey, J.</author><author>Ostby, J.</author></authors></contributors><titles><title>Chronic di-n-butyl phthalate exposure in rats reduces fertility and alters ovarian function during pregnancy in female Long Evans hooded rats</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-

1></alt-periodical><pages>189-
195</pages><volume>93</volume><number>1</number><dates><year>2006</year></dates><isbn>IS
SN 1096-6080
EISSN 1096-0929</isbn><accession-num>16763070</accession-
num><label>673276</label><urls><related-
urls><url>http://dx.doi.org/10.1093/toxsci/kfl035</url></related-urls></urls><electronic-resource-
num>10.1093/toxsci/kfl035</electronic-resource-
num><language>English</language></record></Cite></EndNote>] hypothesized that decreased progesterone production could be mediated through decreased ovarian steroidogenesis, similar to the mechanism for decreased testicular steroidogenesis; or may result from disrupted androgen and INSL3 signaling, since those hormones contribute to maintaining the function of the corpora luteum in pregnant rats. There is also some epidemiologic evidence that DIBP is associated with decreased expression of steroidogenic enzymes in the placenta (CYP11A1, CYP19A1, CYP1B1, 17β-hydroxysteroid dehydrogenase) [ADDIN EN.CITE
<EndNote><Cite><Author>Adibi</Author><Year>2010</Year><RecNum>95</RecNum><DisplayText>(A
dibi et al. 2010)</DisplayText><record><rec-number>95</rec-number><foreign-keys><key app="EN"
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keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Adibi, J.
J.</author><author>Whyatt, R. M.</author><author>Hauser, R.</author><author>Bhat, H.
K.</author><author>Davis, B. J.</author><author>Calafat, A. M.</author><author>Hoepner, L.
A.</author><author>Perera, F. P.</author><author>Tang, D.
/author><author>Williams, P.
L.</author></authors></contributors><titles><title>Transcriptional biomarkers of steroidogenesis and
trophoblast differentiation in the placenta in relation to prenatal phthalate exposure</title><secondary-
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Health Perspect</abbr-1></periodical><alt-periodical><full-title>Environmental Health
Perspectives</full-title><abbr-1>Environ Health Perspect</abbr-1></alt-periodical><pages>291-
296</pages><volume>118</volume><number>2</number><dates><year>2010</year></dates><isbn>I
SSN 0091-6765
EISSN 1552-9924</isbn><accession-num>20123604</accession-
num><label>807122</label><urls><related-
urls><url>http://dx.doi.org/10.1289/ehp.0900788</url></related-urls></urls><electronic-resource-
num>10.1289/ehp.0900788</electronic-resource-
num><language>English</language></record></Cite></EndNote>] with a significantly stronger association in male placentas compared to female in some cases [ADDIN EN.CITE
<EndNote><Cite><Author>Adibi</Author><Year>2017</Year><RecNum>283</RecNum><DisplayText>(
Adibi et al. 2017)</DisplayText><record><rec-number>283</rec-number><foreign-keys><key app="EN"
db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1520292870">283</key></foreign-
keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Adibi, Jennifer
J.</author><author>Buckley, Jessie P.</author><author>Lee, Myoung Keun</author><author>Williams,
Paige L.</author><author>Just, Allan C.</author><author>Zhao, Yaqi</author><author>Bhat, Hari
K.</author><author>Whyatt, Robin M.</author></authors></contributors><titles><title>Maternal
urinary phthalates and sex-specific placental mRNA levels in an urban birth cohort</title><secondary-
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</year><pub-dates><date>April 05</date></pub-dates></dates><isbn>1476-

069X</isbn><label>Adibi2017</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1186/s12940-017-0241-5</url></related-urls></urls><electronic-resource-num>10.1186/s12940-017-0241-5</electronic-resource-num></record></Cite></EndNote>], although results were inconsistent and suggested a possible non-monotonic response.

For male reproductive outcomes, the available studies suggest a sensitivity difference in mice compared to rats; however, there were relatively few mouse studies and all had some concerns for risk of bias and/or sensitivity, so it is difficult to fully delineate species sensitivity differences using the available data. Outcomes associated with the androgen-independent MOA for phthalates (e.g. atrophy of seminiferous cords, germ cell effects) were observed in both species. Conversely, while decreased T was observed in both species, mice did not consistently display the other androgen-dependent outcomes that were observed in rats (e.g. decreased testis weight and AGD). Similar observations on the relative sensitivity of mice and rats have been made for other phthalates, such as DBP [ADDIN EN.CITE <EndNote><Cite><Author>Arzuaga</Author><Year>, in preparation</Year><RecNum>289</RecNum><DisplayText>(Arzuaga et al., in preparation)</DisplayText><record><rec-number>289</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1523359129">289</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Arzuaga, X.</author><author>Walker, T.</author><author>Cooper, G.</author><author>Hotchkiss, A.</author><author>Yost, E.</author></authors></contributors><titles><title>Application of the adverse outcome pathway (AOP) framework to assess species concordance and human relevance of dibutyl phthalate (DBP)-induced male reproductive toxicity </title></titles><dates><year>, in preparation</year></dates><urls></urls></record></Cite></EndNote>]. It has also been found that human xenografts (human fetal testis explants into rodents) are less sensitive than rats to the anti-androgenic effects of phthalates, suggesting an interspecies difference in sensitivity; however, the xenograft results have several factors that limit their interpretation, such as concerns about the developmental stage of the human tissues used in the experiments, and high variability in the results [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In the case of DIBP, results suggest that mice and rats are susceptible to both the androgen-dependent and -independent MOAs, although not all androgen-dependent biomarkers were conserved across species.

The available studies were generally not designed to evaluate female reproductive, liver, or kidney effects, so interpretation of these outcomes is limited. The few studies that evaluated female offspring following gestational exposure provide evidence of morphological effects (displaced ovaries and increased AGD), which could be caused by alterations in hormone signaling analogous to those seen in male offspring. While these observations are compelling, the implications for female fertility are unclear.

A concurrent systematic review of epidemiological studies of phthalates by our colleagues [ADDIN EN.CITE <EndNote><Cite><Author>Radke</Author><Year>, under review</Year><RecNum>291</RecNum><DisplayText>(Radke et al., under review)</DisplayText><record><rec-number>291</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1523359551">291</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Radke, E.;</author><author>Meeker, J.</author><author>Braun, J.</author><author>Cooper, G.</author></authors></contributors><titles><title>Male reproductive effects of phthalate exposure: A systematic review of epidemiological studies </title></titles><dates><year>, under

review</year></dates><urls></urls></record></Cite></EndNote>] found *moderate* evidence of an association between DIBP exposure and reduced testosterone in adult cross-sectional studies, but only *slight* evidence of an association with other male reproductive effects. Comparatively, DBP and several other anti-androgenic phthalates had *robust* evidence of an association with male reproductive toxicity in humans. Given the effects of DIBP in animal models, the low level of evidence for male reproductive toxicity in humans was likely due to the relatively small number and low sensitivity of the available epidemiological studies that evaluated DIBP exposure. Systematic review of the epidemiological literature also provided *moderate* evidence of an association between DIBP exposure and decreased birth size, and *slight* evidence for an association with preterm birth and spontaneous abortion [ADDIN EN.CITE <EndNote><Cite><Author>Radke</Author><Year>, in preparation</Year><RecNum>292</RecNum><DisplayText>(Radke et al., in preparation-a)</DisplayText><record><rec-number>292</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1523360154">292</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Radke, E.</author><author>Braun, J.</author><author>Cooper, G.</author></authors></contributors><titles><title>Female reproductive and developmental effects of phthalate exposure: A systematic review of epidemiological studies</title></titles><dates><year>, in preparation</year></dates><urls></urls></record></Cite></EndNote>].

Epidemiological evidence also suggests that phthalate exposure may be associated with emerging health outcomes such as neurodevelopmental and metabolic toxicity, although no associations with these outcomes were identified for DIBP [ADDIN EN.CITE <EndNote><Cite><Author>Radke</Author><Year>, in preparation</Year><RecNum>293</RecNum><DisplayText>(Radke et al., in preparation-b)</DisplayText><record><rec-number>293</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1523360305">293</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Radke, E.</author><author>Cooper, G.</author><author>Galizia, A.</author><author>Thayer, K.</author></authors></contributors><titles><title>Metabolic, neurodevelopmental, and other emerging health effects of phthalates: A systematic review of epidemiological studies</title></titles><dates><year>, in preparation</year></dates><urls></urls></record></Cite></EndNote>]. Although emerging health outcomes were not the focus of this systematic review of animal studies, we note that the literature search for DIBP identified one behavioral study [ADDIN EN.CITE <EndNote><Cite><Author>Ma</Author><Year>2013</Year><RecNum>70</RecNum><DisplayText>(Ma et al. 2013)</DisplayText><record><rec-number>70</rec-number><foreign-keys><key app="EN" db-id="vpzara2f69w5wjesvxkxzf90efs2ztdrxdps" timestamp="1519058609">70</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Ma, N.</author><author>Liu, S.</author><author>Gao, P.</author><author>Cao, P.</author><author>Xu, H.</author></authors><translated-authors><author>Trusted Translations, Inc</author></translated-authors></contributors><titles><title>Effect of diisobutyl phthalate on learning and memory behavior and apoptosis of hippocampus cells in mice [Translated]</title><secondary-title>Wei Sheng Yan Jiu [Journal of Hygiene Research]</secondary-title><alt-title>Wei Sheng Yan Jiu</alt-title><translated-title><style face="normal" font="default" charset="134" size="100%">邻苯二甲酸二异丁酯对小鼠认知行为及海马神经细胞凋亡的影响</style></translated-title></titles><periodical><full-title>Wei

Sheng Yan Jiu [Journal of Hygiene Research]</full-title><abbr-1>Wei Sheng Yan Jiu</abbr-1></periodical><alt-periodical><full-title>Wei Sheng Yan Jiu [Journal of Hygiene Research]</full-title><abbr-1>Wei Sheng Yan Jiu</abbr-1></alt-periodical><pages>57-60</pages><volume>42</volume><number>1</number><dates><year>2013</year></dates><isbn>ISSN 1000-8020</isbn><accession-num>23596708</accession-num><label>2349624</label><urls></urls><language>English</language></record></Cite></EndNote>], which reported that mice dosed with 1000 mg/kg-day DIBP via oral gavage for 8 weeks had decreased passive avoidance capability and increased apoptosis of hippocampal cells. We performed a preliminary evaluation of this study and found it to have reporting limitations that reduce confidence in the results; however, it does provide suggestive evidence of neurological effects from DIBP exposure.

This systematic review highlighted several ways in which future studies could provide further insight into the mechanisms and characterization of hazards of DIBP exposure. Most of the available studies were targeted at critical exposure windows for male reproductive toxicity, so it would be useful to design chronic or sub-chronic studies that cover critical windows of exposure for other outcomes. For instance, there are currently no one- or multi-generational reproductive toxicity studies available for DIBP; such a study would assess fertility and fecundity, and would provide a point of reference for comparing reproductive effects in animals exposed as adults versus those exposed in utero. Effects on liver, kidney, and cancer are known hazards for other phthalates, so further research is warranted to characterize these outcomes after exposure to DIBP, in addition to evaluating emerging outcomes such as neurodevelopmental toxicity. Additionally, despite the profound effects of DIBP on testosterone observed in males, none of the available studies evaluated hormone production in DIBP-exposed females. It would be useful to evaluate steroidogenesis and INSL3 in female offspring to provide a mechanistic understanding of how each sex is affected by gestational DIBP exposure, and to evaluate altered maternal steroidogenesis and INSL3 as a potential contributing factor to reduced fetal survival.

AUTHOR NOTES

Acknowledgements: This manuscript is dedicated to the memory of Raghu Nath, a biologist at the EPA National Center for Environmental Assessment, who contributed to this evaluation~~assessment~~ of DIBP. We would like to acknowledge Anna Chen, Evangela Matthews, Swati Gummadi, Carolyn Gigot, Mefruz Haque (EPA student services contractors), and Carol Starkey (Oak Ridge Institute for Science and Education) for their assistance in data extraction and visualization for this systematic review.

Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

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Figure 1: Summary of (A) study evaluation and (B) strength of evidence characterization for DIBP animal toxicology studies.

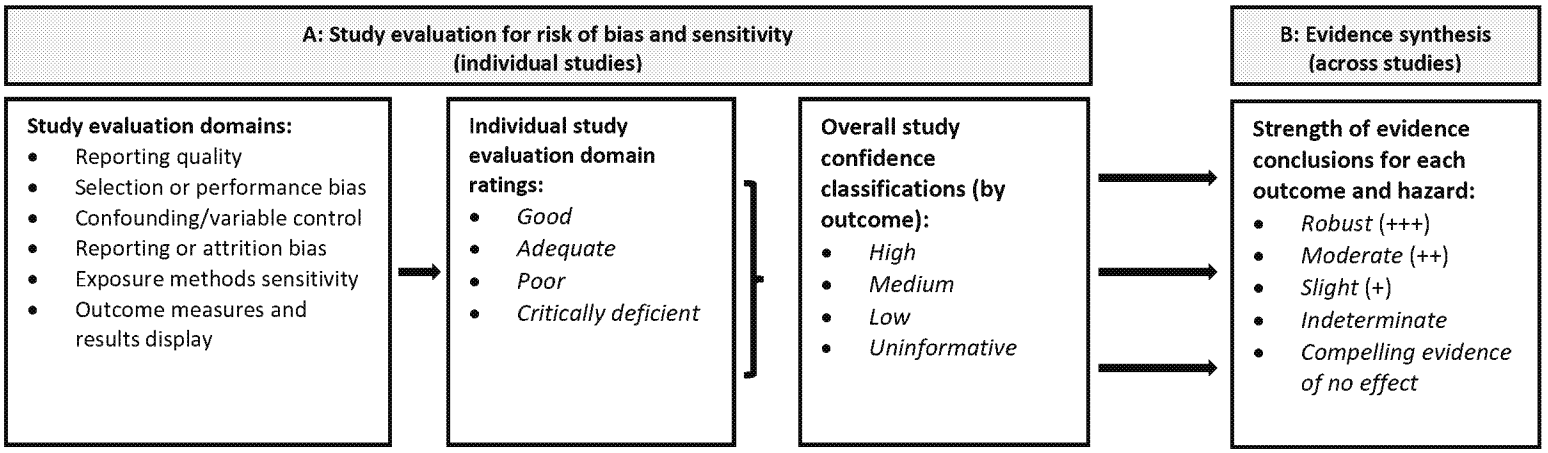
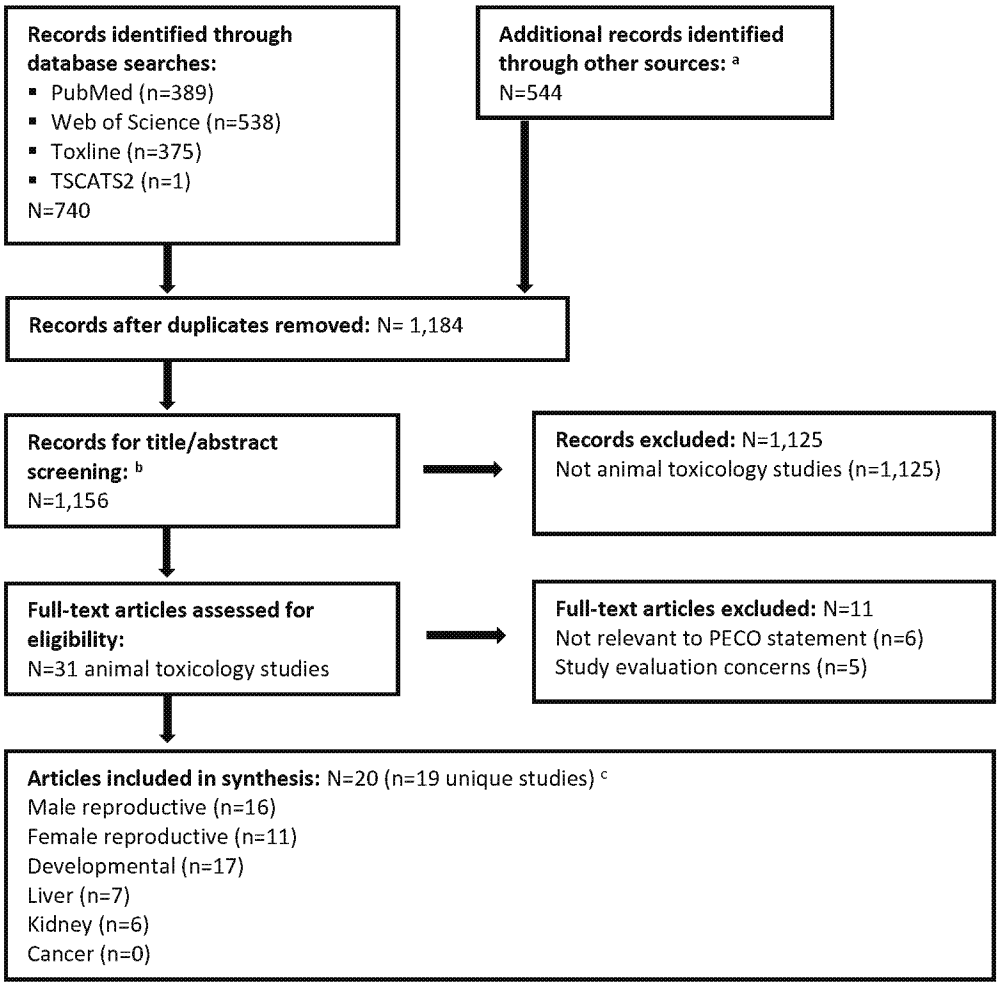


Figure 2: Literature flow diagram for identifying DIBP animal toxicology studies.



[a] Other sources consisted of forward and backward searches, searching citations from key references, manual search of citations from key regulatory documents, references that had been previously identified from an earlier DIBP review effort, and supplementary materials for articles identified during the literature search.

[b] Excludes 28 supplementary materials that were tagged as unique records during the literature search. The main articles from those studies were moved forward for title/abstract screening.

[c] Most studies reported data on multiple hazards; see Table 1.

Table 1: Summary of studies, overall study confidence classifications by outcome[a], and strength of evidence conclusions for each hazard[b]

				Male reproductive [c]				Female reproductive [d]				Developmental [e]			Liver		Kidney	
Author (year)	**Species (strain)**	**Exposure life stage and duration**	**Exposure route**	Testosterone	Morphological development	Reproductive organ weight	Testicular histology and sperm	Morphological development	Maternal body weight	Gestation length	Reproductive organ weight	Survival	Growth	Malformations/ variations	Organ weight	Histopathology	Organ weight	Histopathology
BASF 2007	Rat (Wistar)	GD 6-20	Diet	-	-	-	-	-	H	-	H	H	H	H	-	-	-	-
Borch et al. 2006	Rat (Wistar)	GD 7-19	Gavage	H	H	-	H	H	H	-	-	H	H	-	-	-	-	-
Saillenfait et al. 2006	Rat (Sprague-Dawley)	GD 6-20	Gavage	-	H	-	-	H	H	-	H	H	H	H	-	-	-	-
Howdeshell et al. 2008	Rat (Sprague-Dawley)	GD 8-18	Gavage	H	-	-	-	-	M	-	-	H	-	-	-	-	-	-
Saillenfait et al. 2008	Rat (Sprague-Dawley)	GD 12-21	Gavage	-	H	H	H	H	M	H	-	H	H	-	-	-	-	-
Saillenfait et al. 2017	Rat (Sprague-Dawley)	GD 13-19	Gavage	H	H	-	-	-	H	-	H	H	H	H	-	-	-	-
Furr et al. 2014	Rat (Sprague-Dawley)	GD 14-18	Gavage	H	-	-	-	-	L	-	-	M	-	-	-	-	-	-
Hannas et al. 2012	Rat (Sprague-Dawley)	GD 14-18	Gavage	H	-	-	-	-	L	-	-	M	-	-	-	-	-	-
Hannas et al. 2011	Rat (Sprague-Dawley)	GD 14-18	Gavage	H	-	-	-	-	L	-	-	L	-	-	-	-	-	-
Wang et al. 2017	Mouse (ICR)	GD 0-21; GD 0-PND 21	Diet	M	M	M	M	-	L	-	-	H	M	-	M	-	-	-
Sedha et al. 2015	Rat (Wistar)	PND 21-23; PND 21-40	Gavage	-	-	-	-	M	-	-	M	-	M	-	-	-	-	-
Zhu et al. 2010	Rat (Sprague-Dawley); Mouse (C57Bl/6N)	PND 21-28	Gavage	-	-	L	-	-	-	-	-	-	-	-	-	-	-	-
Oishi and Hiraga 1980a*	Rat (JCL:Wistar)	~PND 35-42	Diet	M	-	M	-	-	-	-	-	-	L	-	-	-	-	-
Oishi and Hiraga 1980b	Rat (JCL:Wistar)	~PND 35-42	Diet	L	-	M	L	-	-	-	-	-	L	-	M	-	M	-
Oishi and Hiraga 1980c*	Mouse (JCL:ICR)	~PND 35-42	Diet	M	-	L	-	-	-	-	-	-	L	-	M	-	M	-
Oishi and Hiraga 1980d	Mouse (JCL:ICR)	~PND 35-42	Diet	M	-	L	-	-	-	-	-	-	L	-	M	-	M	-
Foster et al. 1981, 1982*	Rat (Sprague-Dawley)	"Young"; 6 - day exposure	Gavage	-	-	L	L	-	-	-	-	-	-	-	L	-	L	-
University of Rochester 1953	Rat (albino; strain not reported)	Weaning to 1 month post-weaning	Diet	-	-	-	-	-	-	-	-	-	L	-	M	L	M	L
University of Rochester 1954	Rat (albino; strain not reported)	Weaning to 4 months post-weaning	Diet	-	-	M	-	-	-	-	-	-	L	-	M	L	M	L
				ROBUST (+++)				SLIGHT (+)				ROBUST (+++)			IN.		IN.	
				Strength of evidence conclusion for hazard														

*indicates MIBP study

GD = Gestation day. PND = Postnatal day

[a]High confidence (H; dark green), Medium confidence (M; light green), Low confidence (L; yellow). Dash (-) indicates outcomes that were not included in a study.

[b]Details on the rationale for strength of evidence conclusions are provided in evidence profile tables.

[b]Male reproductive outcomes: Testosterone (testicular production or level measured in testis or serum), morphological development (AGD, nipple retention, hypospadias, cryptorchidism, cleft prepuce, time to puberty), testicular histology and sperm (sperm counts, motility, morphology, histological evaluations of testicular atrophy or azoospermia/oligospermia), reproductive organ weights (testis, epididymides, prostate, seminal vesicles)

[c]Female reproductive outcomes: Maternal body weight (body weight gain during gestation or lactation), gestation length, morphological development (AGD, displaced ovaries, time to puberty), reproductive organ weight (uterus, vagina, ovary)

[d]Developmental outcomes: Survival (fetal viability, fetal mortality, resorptions, pre- or post-implantation loss), growth (pre- or postnatal body weight), malformations/variations (external, skeletal, soft tissue/visceral)

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Table 2: Evidence profile table for male reproductive effects of DIBP or MIBP

*indicates MIBP study

Male Reproductive Effects						
Outcome		**Available studies**	**Factors that increase confidence**	**Factors that decrease confidence**	**Strength of evidence conclusion for outcome**	**Strength of evidence conclusion for overall hazard**
Gestational (F1) Exposure	**Testosterone**	**High confidence:** Borch et al. 2006 Furr et al. 2014 Hannas et al. 2011 Hannas et al. 2012 Howdeshell et al. 2008 Saillenfait et al. 2017 **Medium confidence:** Wang et al. 2017	• Consistency • Dose-response gradient • Effect size • Biological plausibility (support from mechanistic evidence) • Minimal concerns for bias and sensitivity		⊕⊕⊕ ROBUST A dose-related decrease in testicular T levels or production (up to -96% compared to control) was observed in all studies in rats and mice that evaluated this outcome. Several of these studies also demonstrated decreased testicular expression of genes and proteins in the steroidogenesis pathway in both rats and mice, which provides support for biological plausibility.	⊕⊕⊕ ROBUST Supported by consistency and coherence across outcomes, with mechanistic evidence (e.g. decreased testicular expression of steroidogenic enzymes and INSL3 in F1 males) providing support for biological plausibility. The greatest weight of evidence came from gestational exposure studies, whereas postnatal exposure studies were limited by risk of bias and sensitivity concerns.
	Male morphological development	**High confidence:** Borch et al. 2006 Saillenfait et al. 2006 Saillenfait et al. 2008 Saillenfait et al. 2017 **Medium confidence:** Wang et al. 2017	• Consistency within rat studies • Dose-response gradient • Effect size • Biological plausibility • Minimal concerns for bias and sensitivity		⊕⊕⊕ ROBUST All rat studies observed a dose-related increase in effects consistent with decreased testosterone and INSL3, including increased time to puberty, decreased AGD, nipple retention, cryptorchidism, non-scrotal testis, hypospadias, and exposed os penis. No effects on AGD were observed in mice (Wang et al. 2017).	
	Reproductive organ weight	**High confidence:** Saillenfait et al. 2008 **Medium confidence:** Wang et al. 2017	• Dose-response gradient • Biological plausibility	• Few studies	⊕⊕◯ MODERATE Decreased reproductive organ weights were observed in rats (Saillenfait et al. 2008), whereas a consistent trend in testis weight was not observed in mice (Wang et al. 2017).	

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	Testicular histology or sperm evaluation	**High confidence:** Saillenfait et al. 2008 **Medium confidence:** Borch et al. 2006 Wang et al. 2017	• Consistency • Dose-response gradient • Effect size • Biological plausibility		⊕⊕⊕ ROBUST Adverse effects on the testis and/or sperm were observed in rats and mice, including a dose-related increased incidence of pathological lesions of the testis (Borch et al. 2006, Saillenfait et al., 2008), epididymal oligo- or azoopermia (Saillenfait et al. 2008), and decreased sperm concentration and motility (Wang et al. 2017).	
Postnatal (Weanling or Peripubertal) Exposure	**Testosterone**	**Medium confidence:** Oishi and Hiraga 1980a* Oishi and Hiraga 1980c* Oishi and Hiraga 1980d **Low confidence:** Oishi and Hiraga 1980b	• Biological plausibility	• Concerns for bias and sensitivity in some studies • Unexplained inconsistency	◯◯◯ INDETERMINATE A dose-related increase in androgen levels was observed in two rat studies (Oishi and Hiraga 1980ab-c), whereas androgen levels were decreased or not changed in two mouse studies (Oishi and Hiraga 1980c-d).	
	Reproductive organ weight	**Medium confidence:** Oishi and Hiraga 1980a* Oishi and Hiraga 1980b **Low confidence:** Oishi and Hiraga 1980c* Oishi and Hiraga 1980d Foster et al. 1981* U. Rochester 1954 Zhu et al. 2010	• Consistency within rat studies • Biological plausibility • Coherence with gestational exposure studies	• Concerns for bias and sensitivity in some studies	⊕⊕⊕ ROBUST In rats, a dose-related decrease in absolute testis weight was consistently observed (Oishi and Hiraga 1980a-b; Foster at al. 1981; University of Rochester 1954). In weanling mice, Zhu et al. (2010) observed decreased absolute testis weight in the highest dose group. In young adult mice, Oishi and Hiraga (1980c-d) observed increased relative testis weight, which is considered a less reliable metric compared to absolute testis weight.	
	Testicular histology/sperm evaluation	**Low confidence:** Oishi and Hiraga 1980b Foster et al. 1981*	• Consistency • Biological plausibility • Coherence with gestational exposure studies	• Concerns for bias and sensitivity	⊕⊕◯ MODERATE Rats were found to have increased testicular atrophy (Foster et al. 1981) and	

					decreased spermatocytes and spermatogonia (Oishi and Hiraga 1980b).	

Table 3: Evidence profile table for female reproductive effects of DIBP

Female Reproductive Effects						
Outcome		**Available studies**	**Factors that increase confidence**	**Factors that decrease confidence**	**Strength of evidence conclusion for outcome**	**Strength of evidence conclusion for overall hazard**
Gestational (F1) or Postnatal Exposure	**Female morphological development**	**High confidence:** Saillenfait et al. (2006) Saillenfait et al. (2008) Borch et al. 2006 **Medium confidence:** Sedha et al. 2015	• Biological plausibility (coherence with effects observed in males	• Concerns for study sensitivity	⊕◯◯ SLIGHT Increased female AGD was observed, but was not always statistically significant (Borch et al. 2006, Saillenfait et al. 2008). A non-significant increase in displaced ovaries was observed by Saillenfait et al. 2006. There was no effect on the time of vaginal opening following postnatal exposure (Sedha et al. 2015).	⊕◯◯ SLIGHT Based on limited evidence for effects on female AGD and ovary displacement in F1 females following gestational exposure, and for effects on maternal weight gain in F0 females following maternal exposure. There were concerns for study sensitivity because most gestational exposure studies were designed to evaluate male reproductive effects, and the exposure windows may not have been the most sensitive for detecting F1 female or maternal toxicity.
	Reproductive organ weight	**Medium confidence:** Sedha et al. 2015		• Single study	◯◯◯ INDETERMINATE No effects on were observed on uterus, ovary, or vagina weight in prepubescent females.	
Maternal (F0) Exposure	**Maternal body weight**	**High confidence:** Borch et al. 2006 BASF 2007 Saillenfait et al. 2006 Saillenfait et al. 2017 **Medium confidence:** Saillenfait et al. 2008 Howdeshell et al. 2008 **Low confidence:** Furr et al. 2014 Hannas et al. 2011 Hannas et al. 2012 Wang et al. 2017	• Dose-response gradient and minimal concern for bias in the study by BASF 2007	• Concerns for study sensitivity	⊕◯◯ SLIGHT Corrected maternal body weight was significantly decreased in one study (BASF 2007). Otherwise, any effects on maternal weight gain were concurrent with decreased gravid uterine weight or decreased offspring body weight, and therefore appeared to be secondary effects related to fetal toxicity.	

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	Reproductive organ weight	**High confidence:** BASF 2007 Saillenfait et al. 2006 Saillenfait et al. 2017 **Medium confidence:** Sedha et al. 2015	• Minimal concerns for bias	• Indirectness (changes in gravid uterine weight were likely related to fetal toxicity) • Concerns for study sensitivity	○○○ INDETERMINATE Saillenfait et al. 2006 reported decreased gravid uterine weight, which appeared to be a secondary effect related to fetal toxicity. Otherwise, no effects on female reproductive organ weight were observed.	
	Gestation length	**High confidence:** Saillenfait et al. 2008	• Minimal concern for bias	• Single study • Concerns for study sensitivity	○○○ INDETERMINATE Effects on gestation length were not observed in one study that began exposing dams at mid-gestation, which may not be the most sensitive window of exposure for this endpoint	

Table 4: Evidence profile table for developmental effects of DIBP or MIBP

*indicates MIBP study

Developmental effects						
Outcome		**Available studies**	**Factors that increase confidence**	**Factors that decrease confidence**	**Strength of evidence conclusion for outcome**	**Strength of evidence conclusion for overall hazard**
Gestational (F1) Exposure	**Fetal survival**	**High confidence:** BASF 2007 Borch et al. 2006 Howdeshell et al. 2008 Saillenfait et al. 2006 Saillenfait et al. 2008 Saillenfait et al. 2017 Wang et al. 2017 **Medium confidence:** Hannas et al. 2012 Furr et al. 2014 **Low confidence:** Hannas et al. 2011	• Dose-response gradient, effect size, and minimal concern for bias and sensitivity in studies by Saillenfait et al. 2006 and Howdeshell et al. 2008 • Biological plausibility (support from mechanistic evidence for DBP)		⊕⊕⊕ ROBUST A dose-related increase in post-implantation loss was observed in two Sprague-Dawley rat studies that exposed animals from (GD) 6-20 and GD 8-18, respectively (Howdeshell et al. 2008, Saillenfait et al. 2006), but not in Wistar rats or mice exposed for a similar duration. Fetal survival was also not affected in studies that exposed Sprague-Dawley rats for shorter durations in late gestation.	⊕⊕⊕ ROBUST Based on consistent evidence of reduced fetal and postnatal growth across studies, and evidence of reduced fetal survival in Sprague-Dawley rats exposed for longer durations in gestation.
	Fetal growth	**High confidence:** BASF 2007 Borch et al. 2006 Saillenfait et al. 2006 Saillenfait et al. 2017 **Medium confidence:** Wang et al. 2017	• Consistency • Dose-response gradient • Effect size • Minimal concern for bias		⊕⊕⊕ ROBUST A dose-related decrease in fetal growth was reported in all studies used doses of ≥ 500 mg/kg-day, but was not observed in lower dose studies.	
	Fetal morphological development	**High confidence:** BASF 2007 Saillenfait et al. 2006 Saillenfait et al. 2017	• Dose-response gradient and minimal concern for bias and sensitivity in the study by Saillenfait et al. 2006	• Few studies	⊕◯◯ SLIGHT A dose-related increase in external, visceral, and skeletal malformations was reported in Sprague-Dawley rats by Saillenfait et al. 2006, but not in a similar study in Wistar rats by BASF 2007, or at a	

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					a lower dose level by Saillenfait et al. 2017.	
	Postnatal survival	**High confidence:** Saillenfait et al. 2008	• Minimal concern for bias	• Single study	◯◯◯ INDETERMINATE Effects on postnatal survival following gestational exposure were not observed in the single study that evaluated this outcome.	
	Postnatal growth	**High confidence:** Saillenfait et al. 2008 **Medium confidence:** Wang et al. 2017	• Dose-response gradient and minimal concern for bias and sensitivity in the study by Saillenfait et al. 2008	• Few studies	⊕⊕◯ MODERATE A dose-related reduction in postnatal growth was observed in rats exposed during gestation (Saillenfait et al. 2008), but not in a mouse study that tested a single, lower dose of DIBP (Wang et al. 2017).	
Postnatal Exposure	**Postnatal growth**	**Medium confidence:** Sedha et al. 2015 **Low confidence:** Oishi and Hiraga 1980a* Oishi and Hiraga 1980b Oishi and Hiraga 1980c* Oishi and Hiraga 1980d U. Rochester 1953 U. Rochester 1954	• Consistency • Dose-response gradient • Effect size	• Concerns for bias and sensitivity in some studies	⊕⊕◯ MODERATE A dose-related reduction in postnatal and adult growth was observed in all available peripubertal exposure studies in rats and mice. However, in some of the low confidence studies, the reduced growth may have been a secondary effect related to reduced food consumption.	

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