CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of DEHP. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to DEHP, but is not inclusive of the entire body of literature.

Animal inhalation studies are presented in Table 2-1 and Figure 2-2 and animal oral studies are presented in Table 2-2 and Figure 2-3.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects (SLOAELs) are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some

cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of DEHP are indicated in Table 2-2 and Figure 2-3.

A User's Guide has been provided at the end of this profile (Appendix C). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

A comprehensive literature search was conducted to identify epidemiological studies of DEHP and its metabolites, as shown in Figure 3-1 and discussed in Appendix B. The literature search revealed an extensive epidemiological database. For endpoints with large numbers of epidemiological studies, a series of inclusion criteria (Table B-1) were defined to narrow the evaluation to those studies of greatest utility to hazard identification, and only studies meeting the criteria were included in the Toxicological Profile. Selected studies were tabulated and discussed in subsequent sections of this chapter. Recent (since 2011) reviews and systematic reviews of specific health effects, when available, were used to ensure complete coverage of the key literature. However, since urinary metabolites represent the preferred biomarkers for DEHP exposure in human epidemiological studies (Section 3.3.1), and many systematic reviews included studies using metabolite levels in biological media other than urine, the reviews themselves were generally not evaluated in detail.

Additional considerations employed in the assessment of the effects suggested by the epidemiological data include consistency in the direction of effect, number of urinary metabolites measured, and size of study population, as well as corroborating information from animal or mechanistic studies. The epidemiological database for DEHP is extensive, but is largely focused on a small number of endpoints: body weight (body mass index [BMI] and waist circumference), cardiovascular (blood pressure), hepatic (serum lipids), endocrine (diabetes), immunological (allergy and asthma), and reproductive and developmental endpoints.

There are important limitations in the human epidemiological literature for DEHP. In particular, many of the epidemiological studies used a single spot urine sample to assess DEHP exposure. DEHP is rapidly metabolized and excreted, and urinary metabolite levels vary over time within an individual. Thus, a single urine sample may not correlate with long-term exposure patterns unless exposure levels remain very consistent. It is worth noting, however, that exposure to DEHP was probably relatively consistent for many years due to its ubiquitous presence in foods, packaging, and personal care products, until recent efforts to reduce or ban its use were initiated.

As presented in Figure 2-1, most of the available studies on the health effects of DEHP in laboratory animals used oral administration, with a few inhalation studies and two dermal exposure studies identified. The most commonly examined endpoints were developmental, reproductive, body weight, and hepatic. Data presented under individual organ systems are specific to post-pubertal adult animals, while studies evaluating effects following prenatal or early life (pre-pubertal) exposures are considered developmental. Due to the large size of the oral database, oral animal studies were prioritized for efficient review. Studies with inadequate design or reporting and those not meeting certain dose criteria (e.g., high-dose or single-dose studies for well-studied endpoints/durations) were not included in Chapter 2 or Figure 2-1. For example, only acute- and intermediate-duration oral reproductive/ developmental studies that evaluated at least one dose <100 mg/kg/day were included because reproductive/developmental effects have been consistently observed in numerous studies at doses <100 mg/kg/day; for other endpoints, only acute- and intermediate-duration oral studies that evaluated at least one dose <1,000 mg/kg/day were included. Further details can be found in the Prioritization of Animal Data section of Appendix B. For the included studies, the highest NOAELs and all LOAELs can be found in Tables 2-1 and 2-2.

The results of the selected animal studies, along with limited human data, suggest potential associations between DEHP exposure and the following health outcomes:

- **Hepatic effects.** Human data regarding hepatotoxicity are limited and do not show consistent findings. In rodents, high DEHP doses resulted in degenerative and necrotic hepatic changes. At lower DEHP doses, there is evidence of liver enlargement (increased liver weight, hepatocellular hypertrophy) associated with peroxisomal proliferation in rodents; however, these responses are considered adaptive and human relevance is unclear due to association with the nuclear receptors, particularly PPARα (Hall et al. 2012). Thus, doses associated with hepatomegaly were not considered adverse effect levels unless hepatocellular degenerative or necrotic changes or evidence of biliary or other liver cell damage were also present. The lowest doses associated with the liver weight increases and hepatocellular hypertrophy are noted in Tables 2-1 and 2-2 even though the dose levels are considered NOAELs. Studies that evaluated parameters associated with hepatomegaly only (and not clinical chemistry and/or histopathology) were not included in

Tables 2-1 and 2-2 because they were considered inadequate to assess hepatic toxicity; however, these studies are discussed briefly in Section 2.9.

- **Renal effects.** Human data regarding renal effects following DEHP exposure are extremely limited, and do not report consistent findings. In animals, there is some evidence that the kidney is a sensitive target of DEHP toxicity following oral exposure. However, most of the available studies observed kidney damage only at high doses.

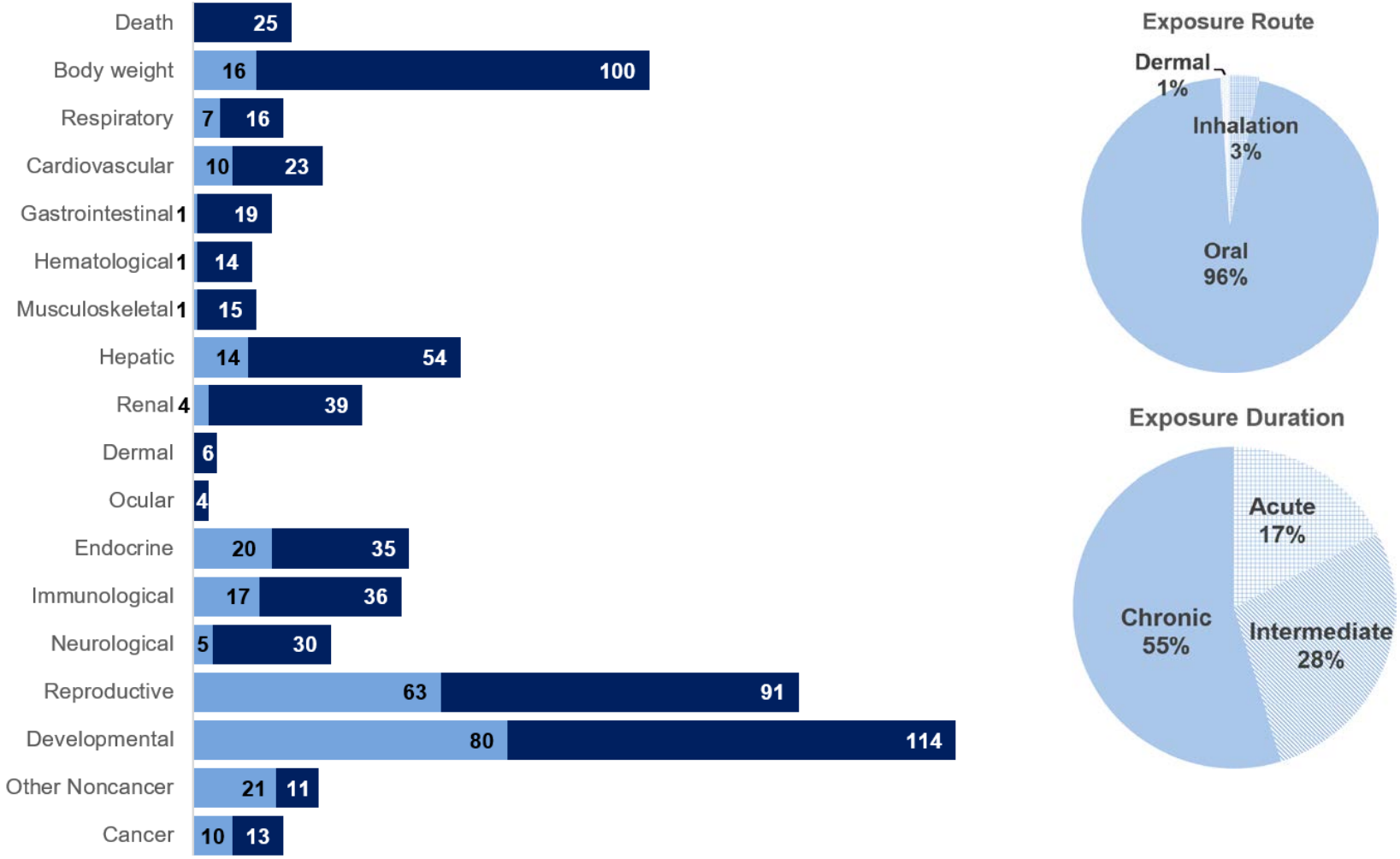
- **Immunological effects.** Human data regarding immunological effects following DEHP exposure are extremely limited. Results from studies evaluating potential associations between prenatal exposure and childhood risk of wheezing or increased IgE were inconsistent. However, some animal studies provide evidence that DEHP is an immune adjuvant in sensitized animals at low exposure levels. The human health relevance of findings from sensitized animals is uncertain in the absence of clear evidence that the immune system is a target of DEHP toxicity in humans or unsensitized animals.

- **Reproductive effects.** Epidemiological studies suggest a potential association between DEHP exposure and decreased serum testosterone and altered sperm parameters in males. Available studies on fertility effects in humans do not indicate an association between DEHP exposure and infertility. In animals, the available oral and inhalation studies provide evidence that the male reproductive system, particularly the testes, is susceptible to DEHP toxicity. Evidence from animal studies indicates decreased male and female fertility at high oral doses.

- **Developmental effects.** Epidemiological studies suggest a potential association between reduced AGD and testicular decent in male infants and prenatal DEHP exposure. In addition, human epidemiological studies provide mixed results for potential relationships between exposure to DEHP and preterm birth, early puberty, and delayed mental and psychomotor development in children. Studies in animals indicate that altered glucose homeostasis and the development of the reproductive system following early life exposure is a particularly sensitive target of DEHP toxicity.

Figure 2-1. Overview of the Number of Studies Examining DEHP Health Effects

Most studies examined the potential body weight, reproductive, and developmental effects of DEHP
Fewer studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 466 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to DEHP – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Concentrations (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effect
ACUTE EXPOSURE									
1	Rat (Wistar) 25 F	10 days GDs 6–15 6 hours/day (WB)	0, 0.6, 3, 21	BW, DX	Develop	3	21		Increased percent of litters with visceral “retardations” (mostly renal pelvis dilatation)
Merkle et al. 1988									
2	Mouse (BALB/c) 8 F	60 minutes (WB)	0.2, 1.2, 2, 19	OF	Resp	2	19		Rapid shallow breathing (35% decrease in tidal volume, 15% increase in respiratory rate) during final 10 minutes of exposure
Larsen et al. 2007 [OVA-sensitized mice]									
INTERMEDIATE EXPOSURE									
3	Rat (Wistar) 27 M, 12 F	4 weeks 5 days/week 6 hours/day (N)	0, 0.6, 3, 63	BW, BC, CS, HE, HP, OW, OF	Bd wt	63			
					Resp	3	63		Transient increases in lung weight, foam cell proliferation, and thickening of alveolar septa
					Cardio	63			
					Hemato	63			
					Musc/skel	63			
					Hepatic	63			Increased relative liver weight at 63 ppm[b]
					Renal	63			
					Endocr	63			
					Immuno	63			
					Neuro	63			
					Repro	63			
Klimisch et al. 1991, 1992									

Table 2-1. Levels of Significant Exposure to DEHP – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Concentrations (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effect
4	Rat (Wistar) 6 M	4 weeks (PNDs 28–56) 6 hours/day 5 days/week (WB)	0, 0.3, 1.6	DX	Develop		0.3[c]		250% increase in plasma testosterone
Kurahashi et al. 2005									
5	Rat (Wistar) 6 M	8 weeks (PNDs 28–84) 6 hours/day 5 days/week (WB)	0, 0.3, 1.6	DX	Develop		0.3[c]		80% increase in plasma testosterone, 30% increase in relative seminal vesicle weight
Kurahashi et al. 2005									
6	Rat (Wistar) 10 F	20 days (PNDs 22–41) 6 hours/day 5 days/week (WB)	0, 0.3, 1.6	DX	Develop		0.3[c]		Vaginal opening and first estrus accelerated by 2-3 days at ≥0.3 ppm; 54% increase in serum estradiol and 167% increase in serum LH at 1.6 ppm
Ma et al. 2006									
7	Rat (Wistar) 10 F	63 days (PNDs 22–84) 6 hours/day 5 days/week (WB)	0, 0.3, 1.6	DX	Develop		0.3[c]		Vaginal opening and first estrus accelerated by 2–3 days at ≥0.3 ppm; irregular estrous cycles and ~10% decrease in PND 84 body weight at 1.6 ppm
Ma et al. 2006									

Table 2-1. Levels of Significant Exposure to DEHP – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Concentrations (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effect
8	Mouse (BALB/c) 9–10 F	14 weeks; 20 minutes/ day 5 days/week for 2 weeks + 1 day/week for 12 weeks (WB)	0, 0.001, 0.006, 0.11, 0.81	BW, OW, IX	Bd wt	0.81			
					Hepatic	0.81			
					Immuno	0.11	0.81		Enhanced immune response to OVA challenge in sensitized animals (350% increase in OVA-specific IgG1)
Larsen et al. 2007 [OVA-sensitized mice]									

[a]The number corresponds to entries in Figure 2-2.
[b]Hepatic effects associated with hepatomegaly (elevated liver weight, hypertrophy, enzyme induction, and/or peroxisome proliferation) are not considered adverse unless hepatocellular degenerative or necrotic changes or evidence of biliary or other liver cell damage were also present (Hall et al. 2012). The lowest doses associated with hepatomegaly endpoints are noted in the LSE tables even though the dose levels are considered NOAELs.
[c]Used to derive an intermediate-duration inhalation minimal risk level (MRL). The LOAEL of 0.3 ppm was adjusted for continuous exposure and was converted to a human equivalency concentration using the default animal:human blood gas partition coefficient ratio of 1 (0.3 ppm x 6 hours/24 hours x 5 days/7days x 1 = 0.05 ppm), and divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for animal to human after dosimetric adjustment, and 10 for human variability), resulting in an MRL of 0.0002 ppm.

BC = serum (blood) chemistry; Bd Wt or BW = body weight; Cardio = cardiovascular; CS = clinical signs; DEHP = di(2-ethylhexyl)phthalate; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); GD = gestational day; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; IX = immunotoxicity; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect level; LSE = levels of significant exposure; M = male(s); Musc/skel = musculoskeletal; (N) = nose-only; Neuro = neurological; NOAEL = no-observed-adverse-effect level; OF = organ function; OVA = ovalbumin; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; (WB) = whole-body

Figure 2-2. Levels of Significant Exposure to DEHP – Inhalation
Acute (≤14 days)

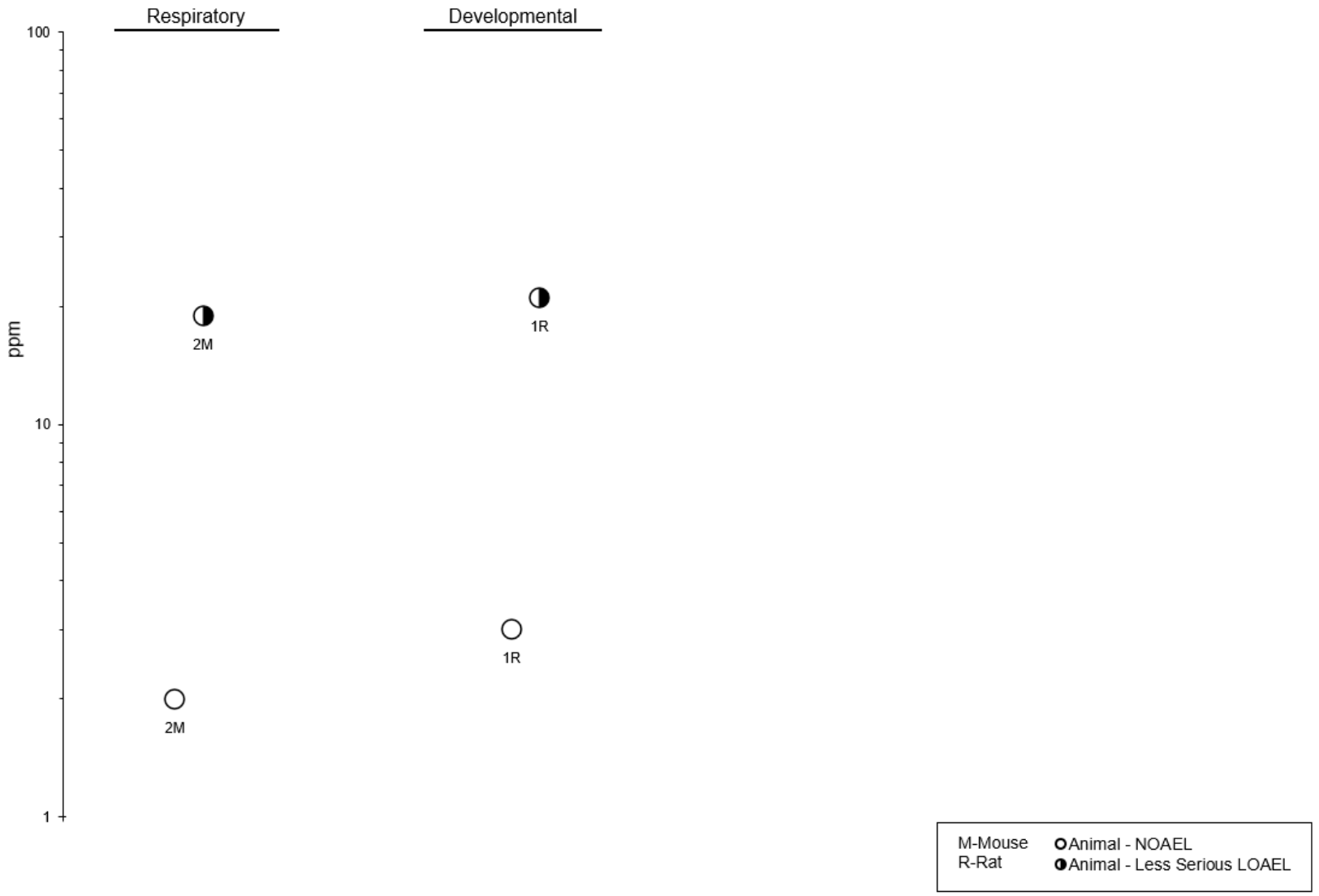


Figure 2-2. Levels of Significant Exposure to DEHP – Inhalation
Intermediate (15–364 days)

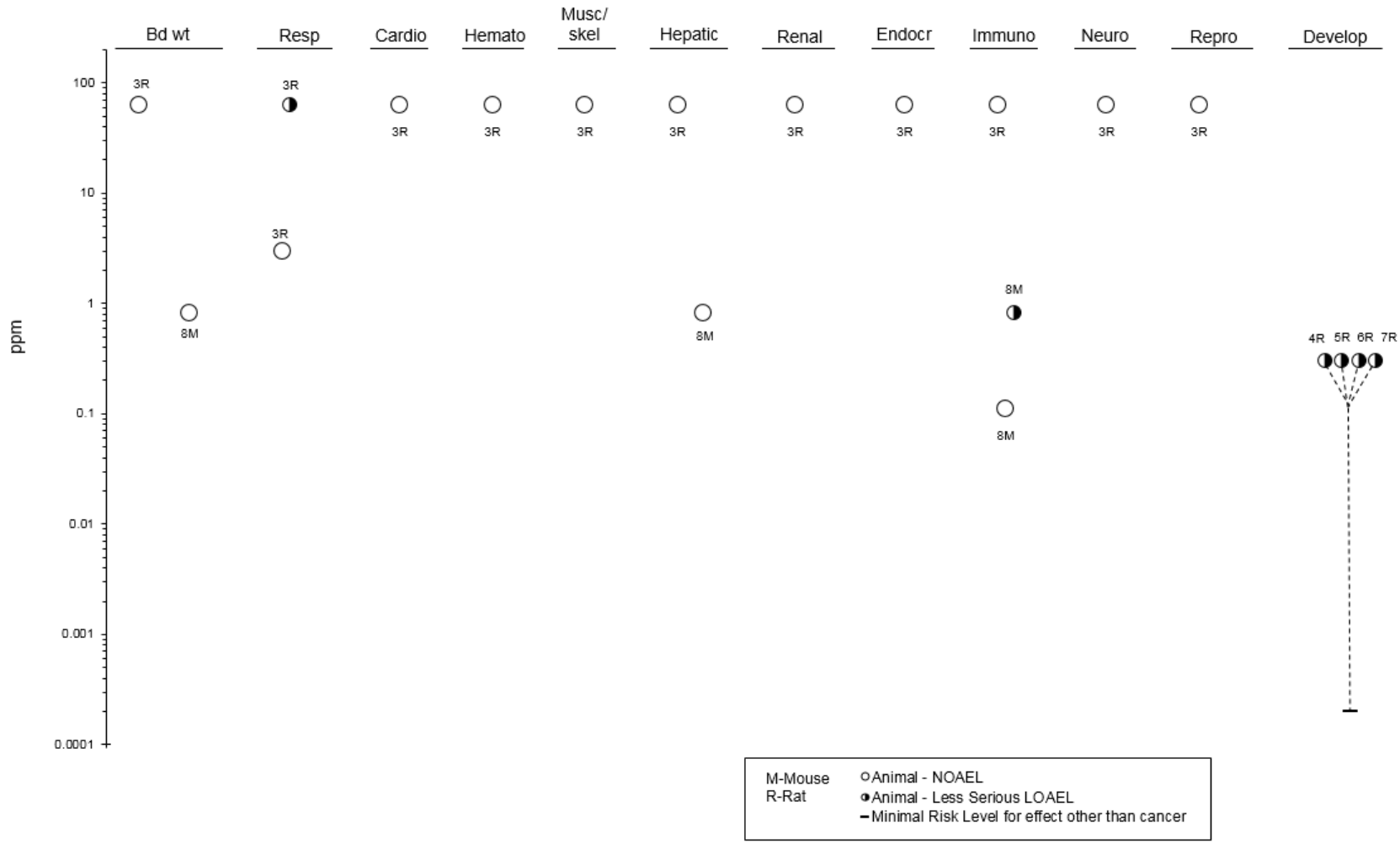


Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE									
1	Monkey (Marmoset) 5 M, 5 F	14 days (GO)	0, 2,000	BC, BI, BW, HE, HP, OW	Hemato	2,000			
					Hepatic	2,000			
					Renal	2,000			
					Neuro	2,000			
					Repro	2,000			
ICI Americas Inc. 1982; Rhodes et al. 1986									
2	Monkey (Cynomol-gus) 4 M	14 days (G)	0, 500	CS, BC, BI, BW, HE, HP, OW, UR	Develop	500			
Pugh et al. 2000 [Exposure prior to sexual maturity.]									
3	Rat (Long-Evans) 10 M	14 days PNDs 21–34 (GO)	0, 1, 10, 100, 200	DX	Develop	10	100		45% reduction in basal and LH-stimulated testosterone production in Leydig cells
Akingbemi et al. 2001									
4	Rat (Long-Evans) 10 M	14 days PNDs 35–48 (GO)	0, 1, 10, 100, 200	DX	Develop	1	10		40% reduction in basal and LH-stimulated testosterone production in Leydig cells
Akingbemi et al. 2001									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
5	Rat (Fischer-344) 4 M, 4 F	1 week (F)	M: 0, 85, 530, 1,100 F: 0, 86, 570, 940	BC, BI, BW, EA, FI, HP, OW	Bd wt	1,100			
					Hepatic	85	530		Decreased serum lipids, increased absolute and relative liver weight, enzyme induction at ≥530 mg/kg/day; increased hepatocellular hypertrophy in males at 1,100 mg/kg/day
					Musc/skel	1,100			
					Renal	1,100			
					Endocr	1,100			
					Immuno	1,100			
					Neuro	1,100			
					Repro	1,100			
Astill et al. 1986									
6	Rat (Fischer-344) 8 F	Once (GO)	0, 150, 500, 1,500, 5,000	HP, OW	Hepatic	500	1,500		Centrilobular necrosis or inflammation at ≥1,500 mg/kg/day; increased liver weight and hepatocellular hypertrophy at all doses[b]
					Endocr	5,000			
					Immuno	5,000			
Berman et al. 1995									
7	Rat (Fischer-344) 8 F	14 days (GO)	0, 50, 150, 500, 1,500	BW, HP, OW	Hepatic	500	1,500		Centrilobular necrosis and inflammation at ≥1,500 mg/kg/day; Increased relative liver weight and hepatocellular hypertrophy at ≥150 mg/kg/day[b]
					Endocr	1,500			
					Immuno	1,500			
Berman et al. 1995									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
8	Rat (Sprague-Dawley) 6 M	5 days PNDs 3–7 (G)	0, 60, 300, 600	DX	Develop		60	600	LOAEL: 16% decrease in absolute testes weight, mitotic alterations in gonocytes; >20% decrease in absolute and relative testes weight and polynucleated gonocytes at ≥300 mg/kg/day; increased liver weight[b] at ≥300 mg/kg/day Serious LOAEL: 21% decrease in body weight, Sertoli cell apoptosis
Camacho et al. 2020 [Vehicle was Intralipid 20%.]									
9	Rat (Sprague-Dawley) 8–10 F	10 days GD 12– PND 0 (GO)	0, 10, 100, 750	BW, DX	Bd wt	100		750	103% decrease in maternal weight gain during exposure period (dams lost weight)
					Develop	10	100	750	~7% decrease in pup birth weight at 100 mg/kg/day; 12% decrease in pup birth weight, increased thickness of alveolar septa, and increased interstitial lung tissue proportion in offspring at 750 mg/kg/day
Chen et al. 2010									
10	Rat (Fischer-344) NS F	7 days PNDs 1–21 (GO)	0, 500, 1,000, 2,500, 5,000	DX, HP, OW	Death			5,000	25% maternal mortality
Cimini et al. 1994									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
11	Rat (Sprague-Dawley) 6–10 M	5 days PNDs 6–10, 14–18, 16–20, 21–25, or 42–46, (GO)	0, 10, 100, 1,000, 2,000	LE, DX	Death			1,000	68% mortality in rats treated on PNDs 14–18; 98% mortality in rats with initiation at or before PND 21 with 2,000 mg/kg/day
					Develop	100			
Dostal et al. 1987									
12	Rat (Sprague-Dawley) 6–10 M	5 days PNDs 86–90 (GO)		BI, OW	Renal	2,000			
Dostal et al. 1987									
13	Rat (Sprague-Dawley) 7–10 M	5 days PNDs 6–10 (GO)	0, 10, 100, 1,000, 2,000	DX	Develop	100	1,000		Reduced absolute and relative testes weight and number of Sertoli cells
Dostal et al. 1988									
14	Rat (Sprague-Dawley) 7–10 M	5 days PNDs 14–18 (GO)	0, 10, 100, 1,000, 2,000	DX	Develop	100	1,000		Reduced testes weight; reduced number of spermatocytes
Dostal et al. 1988									
15	Rat (Sprague-Dawley) 7–10 M	5 days PNDs 21–25 (GO)	0, 10, 100, 1,000, 2,000	DX	Develop	100	1,000		Decreased testicular weight; reduced number of spermatocytes
Dostal et al. 1988									
16	Rat (Sprague-Dawley) 7–10 M	5 days PNDs 42–46 (GO)	0, 10, 100, 1,000, 2,000	DX	Develop	100	1,000		Reduced absolute and relative testicular weight; reduced number of spermatids and spermatocytes
Dostal et al. 1988									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
17	Rat (Sprague-Dawley) 7–10 M	5 days PNDs 86–90 (GO)	0, 10, 100, 1,000, 2,000	BI, HP, OW	Repro	100	1,000		Reduced number of spermatids and spermatocytes; decreased testicular zinc
Dostal et al. 1988									
18	Rat (Sprague-Dawley) 6–8 F	5 days GDs 14–18 (GO)	0, 100, 300, 600, 900	DX	Develop		100	300	21% decrease in fetal testicular testosterone production; ≥67% decrease in fetal testosterone production at ≥300 mg/kg/day
Furr et al. 2014; Hannas et al. 2011									
19	Rat (Long-Evans) 19–38 M	14 days PNDs 21–34 (GO)	0, 10, 500	DX	Develop	10	500		Decreased testes weight, serum testosterone, and Leydig cell testosterone production
Ge et al. 2007									
20	Rat (Long-Evans) 6 M	7 days (GO)	0, 10, 750	BW, HP	Bd wt	750			
					Repro		10		Increased Leydig cell number in testes
Guo et al. 2013									
21	Rat (Long-Evans) 6 M	11 days (GO)	0, 10, 750	BC, EA, HP	Repro		10		Increased Leydig cell proliferation following EDS elimination of Leydig cells
Guo et al. 2013									
22	Rat (Sprague-Dawley) 3–6 F	5 days GDs 14–18 (GO)	0, 100, 300, 500, 625, 750, 875	BW, DX	Bd wt	500		625	>50% decrease in maternal body weight gain
					Develop	100	300		Decreased fetal testicular testosterone production
Hannas et al. 2011									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
23	Rat (Wistar) 3–6 F	5 days GDs 14–18 (GO)	0, 100, 300, 500, 625, 750, 875	BW, DX	Bd wt	500		625	>30% decrease in maternal body weight gain
					Develop	100	300		Decreased fetal testicular testosterone production
Hannas et al. 2011									
24	Rat (Wistar) 9–10 F	9 days GDs 6–15 (GO)	0, 40, 200, 1,000	BW, CS, OW, RX, DX	Bd wt	1,000			
					Renal	200	1,000		Increased relative maternal kidney weight
					Repro	200		1,000	Increased resorptions and post-implantation loss; vaginal hemorrhage in 2/9 dams; decreased maternal uterine weight
					Develop	200		1,000	34% decrease in the number of live fetuses/dam; increased number of fetuses/litter with malformations (70.1%), variations (80.2%), and retardations (58.3%)
Hellwig et al. 1997									
25	Rat (Sprague-Dawley) 4 F	11 days GDs 8–18 (GO)	0, 100, 300, 600, 900	BW, DX	Bd wt	900			
					Develop	100	300	900	20% decreased in fetal testicular testosterone production; 80% decrease in fetal testosterone production at 900 mg/kg/day
Howdeshell et al. 2008									
26	Rat (Sprague-Dawley) 6 M	10 days (G)	0, 40, 400	CS, BW, OW	Bd wt	400			
					Renal		40		11% increase in kidney weight (castrated rats without testosterone supplementation)
					Repro	400			
Kim et al. 2018b [Hershberger assay; castrated rats with and without testosterone supplementation]									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
27	Rat (Sprague-Dawley) 8 F	7 days GDs 13–19 (GO)	0, 10, 100	DX	Develop		10	100	Leydig cell clustering in fetal testes at ≥10 mg/kg/day; dysgenic seminiferous cords and decreased fetal testicular testosterone production at 100 mg/kg/day
Klinefelter et al. 2012									
28	Rat (Sprague-Dawley) 5 M	2 weeks (GO)	0, 25, 100, 250, 1,000	EA, HP, OW	Hepatic	1,000			Increased relative liver weight and peroxisomal markers at ≥100 mg/kg/day; enzyme induction and increased peroxisomal proliferation at higher doses[b]
Lake et al. 1984									
29	Rat (Sprague-Dawley) 6 M	10 days (GO)	0, 20, 100, 500	BC, BW, CS, OW	Bd wt	500			
					Renal	500			
					Endocr	500			
					Repro		20		Decreased ventral prostate weight at ≥20 mg/kg/day; decreased seminal vesicle weight and increased serum LH at ≥100 mg/kg/day; decreased LABC muscle weight at 500 mg/kg/day
Lee and Koo 2007 [Hershberger assay; castrated rats supplemented with testosterone]									
30	Rat (Sprague-Dawley) 5 M	Once PND 3 (GO)	0, 20, 100, 200, 500	DX	Develop	20	100		Multinucleated gonocytes and reduced Sertoli cell proliferation on PND 4
Li et al. 2000									
31	Rat (Long-Evans) 8 M	14 days (GO)	0, 10, 750	BC, RX	Bd wt	750			
					Repro		10		200 and 140% increase in Leydig cell number and proliferation,

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
									respectively (following EDS elimination of Leydig cells)
Li et al. 2012a									
32	Rat (Sprague-Dawley) 3 F	8 days GD 14–PND 0 (GO)	0, 20, 50, 100, 300, 750	DX	Develop	50 M	100 M		Decreased serum testosterone and aldosterone at ≥100 mg/kg/day; reduced adrenal weight at 750 mg/kg/day
						100 F	300 F		Decreased serum estradiol and increased serum aldosterone at ≥300 mg/kg/day; reduced adrenal weight at 750 mg/kg/day
Martinez-Arguelles et al. 2011 [Effects were measured in adult (PND 60) offspring.]									
33	Rat (Sprague-Dawley) NS F	8 days GD 14–PND 0 (GO)	0, 300	DX	Develop		300		Decreased serum aldosterone and mild decreases in systolic blood pressure at PND 200; decreased nighttime locomotor activity at PNDs 60 and 200
Martinez-Arguelles et al. 2013									
34	Rat (Fischer-344) 8 F	Once (GO)	0, 150, 500, 1,500, 5,000	CS, NX	Neuro	1,500	5,000		Signs of general debilitation
Moser et al. 1995									
35	Rat (Fischer-344) 8 F	14 days (GO)	0, 50, 150, 500, 1,500	CS, NX	Neuro	1,500			
Moser et al. 1995									
36	Rat (Fischer-344) 10 F	10 days (GO)	0, 50, 100, 150, 200	CS, BW, NX	Bd wt	200			
					Neuro	200			
Moser et al. 2003									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
37	Rat (Fischer-344) 5 M, 5 F	14 days (F)	M: 0, 670, 1,300, 2,700, 5,700, 12,000 F: 0, 730, 1,500, 3,000, 6,200, 12,000	LE	Death			12,000	2/5 males and 4/5 females died
NTP 1982									
38	Rat (Wistar) 6 F	13 days GDs 9–21 (GO)	0, 1, 10, 100	DX	Develop		1[c]	10	Altered glucose homeostasis at ≥1 mg/kg/day (16–20% increase in blood glucose, 21–22% decrease in serum insulin); 12–21% decreased body weight and increased adipose tissue at ≥10 mg/kg/day in adult offspring
Rajesh and Balasubramanian 2014									
39	Rat (Fischer-344) 4–7 M	1 week (F)	0, 500, 4,000	BC, EA, OW	Hepatic		500		Decreased serum triglycerides at ≥500 mg/kg/day; decreased serum cholesterol, increased relative liver weight, markers of peroxisomal proliferation at 4,000 mg/kg/day
Reddy et al. 1976									
40	Rat (Sprague-Dawley) 8–12 F	8 days GDs 12–19 (GO)	0, 50, 625	DX	Develop		50	625	28% decrease in fetal testosterone production; 85% decrease in fetal testosterone production at 625 mg/kg/day
Saillenfait et al. 2013									
41	Rat (Wistar) 10 NS	Once (G)	≤79,500	CS, BW, LE	Death			30,600	LD_{50}; 8/10 died at 79,500 mg/kg/day
Shaffer et al. 1945									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
42	Rat (Wistar) 8 M	10 days (GO)	0, 4, 20, 100, 200, 400, 600, 800, 1,000	BW, OW	Bd wt Repro	1,000 20	 100		 Decreased LABC muscle weight at ≥100 mg/kg/day; decreased prostate weight at ≥200 mg/kg/day; decreased seminal vesicles weight at ≥400 mg/kg/day
Stroheker et al. 2005 [Hershberger assay; castrated rats supplemented with testosterone]									
43	Rat (Sprague-Dawley) 8 F	11 days GDs 11–21 (GO)	0, 10, 100, 500	DX	Develop		10	500	Effects at PNDs 13–63: 14–16% decrease in sperm concentration, viability, and motility; decreased AGD at 100 mg/kg/day; increased nipple retention, hypospadias, and cryptorchidism at 500 mg/kg/day Effects at GD 21: 14% decrease in fetal body weight; decreased serum testosterone and LH at 500 mg/kg/day
Vo et al. 2009a									
44	Rat (Sprague-Dawley) NS F	8 days GD 14–PND 0 (GO	0, 0.1, 10	DX	Develop	10			
Walker et al. 2020 [Reproductive function was assessed in adult male offspring.]									
45	Rat (Sprague-Dawley) 10 F	4 days ~PNDs 26–30 (GO)	0, 20, 200, 2,000	DX	Develop	2,000			
Zacharewski et al. 1998 [immature ovariectomized rats]									
46	Rat (Sprague-Dawley) 10 F	4 days (GO)	0, 20, 200, 2,000	BW, OW	Bd wt Repro	2,000 2,000			

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Zacharewski et al. 1998 [mature ovariectomized rats]									
47	Rat (Sprague-Dawley) 6 M	14 days PNDs 22–35	0, 50, 150, 450	DX	Develop	50	150	450	Decreased absolute (-30%) and relative (-23%) testis weight, decreased thickness and vacuolization of the seminiferous epithelium at ≥150 mg/kg/day; severe vacuolization of seminiferous epithelium and lack of spermatids in tubules at 450 mg/kg/day
Zhang et al. 2018a									
48	Mouse (CD-1) NS F	10 days GD 11 – PND 0	0, 0.2, 500, 750 (micro-pipette)	DX	Develop		0.2		Increased anxiety (48% fewer entries into center of an open field); impaired memory, 26–38% fewer hippocampal pyramidal neurons, and altered histology of pyramidal neurons at ≥500 mg/kg/day
Barakat et al. 2018									
49	Mouse (CD-1) 9–20 F	10 days GDs 9–18	0, 0.0005, 0.001, 0.005, 0.5, 50, 500 (micro-pipette)	BC, DX, RX	Repro Develop	500 0.5	 50		Decreased fetal testes weight
Do et al. 2012									
50	Mouse (A/J) 10 M	2 weeks (F)	0, 12.3, 125	BW, FI, WI, HP, RX	Bd wt Repro	125 	 12.3		Sertoli cell vacuolation at ≥12.3 mg/kg/day; germ cell sloughing in seminiferous tubules at 125 mg/kg/day
Kitaoka et al. 2013									
51	Mouse (C57BL/6) 4 M	5 days (G)	0, 4, 40, 400, 2000	BC	Other noncancer	400	2,000		Impaired glucose homeostasis (increased blood glucose with glucose challenge)

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Lee et al. 2019a									
52	Mouse (C57BL/6) 10 F	6 days GDs 12–17 (GO)	0, 100, 200, 500	DX	Develop		100		Increased incidence of hypospadias and decreased AGD on GD 19 at ≥100 mg/kg/day; decreased anterior urethra length at ≥200 mg/kg/day
Liu et al. 2008									
53	Mouse (CD-1) 10 F	9 days GDs 11–19 (GO)	0, 25, 100	BW, DX, FI, HP, OW	Bd wt	100			
					Hepatic	100			
					Repro	100			
					Develop		25		Reversible liver lesions in PND 21 offspring (pyknotic nuclei, hepatocyte vacuolization)
Maranghi et al. 2010									
54	Mouse (ddY-Slc) 3–8 F	Once GD 6, 7, 8, 9, or 10 (G)	0, 50, 100, 1,000, 2,500, 5,000, 7,500, 10,000, 30,000	BW, DX	Develop	50		100	11.2% fetal lethality
Nakamura et al. 1979; Tomita et al. 1982a; Yagi et al. 1980									
55	Mouse (B6C3F1) 5 M, 5 F	14 days (F)	M: 0, 1,200, 2,400, 4,900, 10,000, 20,000 F: 0, 1,400, 2,700, 5,300, 11,000, 23,000	DX, LE	Death			11,000 F	4/5 died at 11,000 mg/kg/day, 5/5 died at 20,000 mg/kg/day
								20,000 M	5/5 died
NTP 1982									
56	Mouse (C57BL/6) 6 M	10 days (F)	0, 180, 360	BW, OW, IX	Bd wt	360			
					Immuno	360			
Sasaki et al. 2003									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
57	Mouse (C57BL/6) 8–18 F	10 days GDs 7–16 (GO)	0, 5, 250, 500	CS, BW, RX, DX	Bd wt	500			
					Repro	5		250	Increased resorptions, 67% and 94% decrease in number of pups/litter at 250 and 500 mg/kg/day, respectively
					Develop	5		250	<50% fetal survival; increased total malformations, limb malformations, exencephaly; ~10% decrease in surviving female fetal weight
Ungewitter et al. 2017 [Females were mated with unexposed B6129S4 males.]									
58	Mouse (ICR) 8–10 F	14 days GDs 0–14 (GO)	0, 50, 200	BC	Repro		50		240% increase in serum progesterone
Zhang et al. 2020b									
59	Hamster (Syrian) 5 M	2 weeks (GO)	0, 25, 100, 250, 1,000	EA, HP, OW	Hepatic	1,000			Increased relative liver weight and peroxisomal proliferation at 1,000 mg/kg/day[b]
Lake et al. 1984									
60	Rabbit (NS) 4–5 M	7 days (GO)	0, 2,000	LE	Death			2,000	50% died
Parmar et al. 1988									
61	Rabbit (NS) NS	Once (G)	NS	CS, BW	Death			33,900	LD_{50}
Shaffer et al. 1945									
INTERMEDIATE EXPOSURE									
62	Monkey (Marmoset) 4 M, 4 F	13 weeks (GO)	0, 100, 500, 2,500	BC, BI, BW, CS, EA, GN, HE, HP, OW	Bd wt	2,500			
					Resp	2,500			
					Cardio	2,500			
					Gastro	2,500			

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Hemato	2,500			
					Musc/skel	2,500			
					Hepatic	2,500			
					Renal	2,500			
					Dermal	2,500			
					Ocular	2,500			
					Endocr	2,500			
					Repro	2,500			
Kurata et al. 1998									
63	Monkey (Cynomol-gus) 3 M, 3–4 F	28 days (GO)	0, 1,000	BC, EA, HE, HP, OW	Hemato	1,000			
					Hepatic	1,000			
					Renal	1,000			
Satake et al. 2010									
64	Rat (Fischer-344) 24 M	60 days (F)	0, 17.5, 69.2, 284.1, 1,156.4	BW, FI, BC, OW, HP, OW	Bd wt	284.1	1,156.4		10–15% decrease in body weight; no change in food consumption
					Hepatic	17.5	69.2		Decreased serum lipids at ≥69.2 mg/kg/day; increased liver weight at ≥284.1 mg/kg/day
					Repro	284.1	1,156.4		Testicular atrophy, decreased reproductive organ weights, sperm decrements and abnormalities
Agarwal et al. 1986									
65	Rat (Long-Evans) 10 M	28 days PNDs 21–48 (GO)	0, 1, 10, 100, 200	DX	Develop	1	10		Increased serum testosterone and LH; increased Leydig cell testosterone production
Akingbemi et al. 2001									
66	Rat	28 days PNDs 62–89	0, 1, 10, 100, 200	BC, BW, HP, RX	Bd wt	200			
					Repro	200			

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
	(Long-Evans) 10 M	(GO)							
Akingbemi et al. 2001									
67	Rat (Long-Evans) 10 M	28 days PNDs 21–48 (GO)	0, 10, 100	DX	Develop		10		Increased serum estradiol and Leydig cell estradiol production
Akingbemi et al. 2004									
68	Rat (Long-Evans) 10 M	70 days PNDs 21–90 (GO)	0, 10, 100	DX	Develop		10		Increased serum testosterone and LH, decreased Leydig cell testosterone and estradiol production, Leydig cell proliferation
Akingbemi et al. 2004									
69	Rat (Long-Evans) 10 M	100 days PNDs 21–120 (GO)	0, 10, 100	DX	Develop		10		Leydig cell proliferation at ≥10 mg/kg/day; increased serum testosterone and decreased Leydig cell testosterone production at 100 mg/kg/day
Akingbemi et al. 2004									
70	Rat (Wistar) 11–16 F	37 days GD 6–PND 21 (GO)	0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405	BW, RX, DX, OW	Bd wt	405			
					Renal	405			
					Endocr	405			
					Immuno	405			
					Neuro	405			
					Repro	405			
					Develop	5	15		Delayed PPS and vaginal opening and decreased sperm

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
									production at ≥15 mg/kg/day; testicular lesions at ≥135 mg/kg/day; increased nipple retention and decreased AGD in males and increased tertiary atretic follicles in females at 405 mg/kg/day
Andrade et al. 2006a, 2006b, 2006c; Grande et al. 2006, 2007									
71	Rat (Long-Evans) 12 F	42 days GD 1–PND 21 (W)	0, 3, 30	BW, DX, RX	Bd wt	30			
					Repro	30			
					Develop			3	PNDs 21–56: permanent testes damage and reversible liver and kidney damage at ≥3 mg/kg/day, impaired learning in females at 30 mg/kg/day
Arcadi et al. 1998									
72	Rat (Fischer-344) 5 M, 5 F	3 weeks (F)	M: 0, 75, 470, 950 F: 0, 79, 490, 930	BC, BI, BW, EA, FI, HP, OW	Bd wt	950			
					Musc/skel	950			
					Hepatic		75		Decreased serum lipids, increased liver weight, enzyme induction at ≥75 mg/kg/day; hepatocellular hypertrophy and peroxisomal proliferation at 470 mg/kg/day
					Renal	470	930		Increased absolute and relative kidney weight
					Endocr	950			
					Immuno	950			
					Neuro	950			
					Repro	950			
Astill et al. 1986									
73	Rat				Bd wt	400			

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
	(Wistar) 6 M	4 weeks (GO)	0, 100, 200, 400	BW, BC, BI, HP, OW	Hepatic		100		Histopathological changes (increased congestion, mononuclear cell infiltration, sinusoidal degeneration), 18-21% increase in serum ALT and AST, 57% increase in absolute liver weight
					Renal		100		Histopathological changes (increased glomerular degeneration, congestion and mononuclear cell infiltration), 25% increase in absolute kidney weight; increased serum urea at ≥200 mg/kg/day
					Other noncancer		100		10% increase in serum glucose
Aydemir et al. 2018									
74	Rat (Fischer-344) 5 M, 5 F	21 days (F)	M: 0, 11, 105, 667, 1,224, 2,101 F: 0, 12, 109, 643, 1,197, 1,892	BC, BI, BW, FI, HP, OW	Bd wt	1,224			38–44% decrease in body weight and 48–60% decreased in food consumption at ≥1,892 mg/kg/day
					Hepatic	11	105		Reduced serum lipids at ≥105 mg/kg/day; increased liver weight and peroxisome proliferation, decreased cytoplasmic basophilia, increased cytoplasmic eosinophilia at ≥643 mg/kg/day
					Renal	2,101			
					Repro	1,224 M		2,101 M	Decreased testicular weight and testicular atrophy
Barber et al. 1987; CMA 1986 [Female reproductive organs were not assessed.]									
75	Rat (Sprague-Dawley)	24 weeks (3-generation)	0.1, 0.58, 1.7, 5.9, 17, 57, 447, 659	BW, FI, OW, HP, RX, DX	Bd wt	57 M	447 M		10–19% decreased F1/F2 body weight; no change in food consumption

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
	17 M, 17 F	6 weeks premating through 3 weeks post-weaning of 3rd litter (F)				447 F	659 F		12–24% decreased F0/F1 body weight; no change in food consumption
					Hepatic	659			Increased liver weight and hepatocellular hypertrophy in all generations at ≥57 mg/kg/day[b]
					Renal	57	447		Increased kidney weight, medullary mineralization, and tubular dilation in parental animals
					Endocr	447 M	659 M		Increased relative adrenal gland weight in parental males; adrenal cortical vacuolation in F0 males
						659 F			
					Neuro	659			
					Repro	5.9 M	17 M	659 M	Reproductive tract malformations in F1 and F2 adults at ≥17 mg/kg/day; male reproductive organ and sperm damage at higher doses; decreased F1/F2 pregnancy rate at 447 mg/kg/day; complete loss of F1 male fertility at 659 mg/kg/day
						659 F			
					Develop	57	447		Decreased birth weight in F2 pups at ≥47 mg/kg/day and F1 pups at 659 mg/kg/day; decreased AGD in males in all generations; delayed maturation in all generations

Blystone et al. 2010; NTP 2005 [3-generation, continuous breeding study with cross-over mating]

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
76	Rat (Wistar) 8 F	15 days GDs 7–21 (GO)	0, 10, 30, 100, 300	DX	Develop	30	100		Increased gonocyte number and centralized and multinucleated germ cells in fetal testes at ≥100 mg/kg/day; Leydig cell clustering, Sertoli cell vacuolization, decreased testicular testosterone content and production in fetal testes at 300 mg/kg/day
Borch et al. 2006									
77	Rat (Sprague-Dawley) 6 M	21 days PNDs 3–23 (G)	0, 60, 300, 600	DX	Develop		60	600	LOAEL: decreased seminiferous tubule diameter, 15–24% decrease in testis and seminal vesicle weight; decreased testicular tissue area, germinal cell depletion, 15% decrease in kidney weight and renal tubule degeneration Serious LOAEL: 27% decrease in body weight, developmental malformations in the lung parenchyma, hepatocellular hypertrophy and increased liver weight[b]
Camacho et al. 2020 [Vehicle was Intralipid 20%.]									
78	Rat (Wistar) 3 F	42 days GD 1–PND 21 (W)	0, 3, 30	DX	Develop	3	30		Decreased serum FSH and reduced absolute testis weight on PND 30 in male offspring
Carbone et al. 2010									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
79	Rat (Wistar) 3 F	36 days GD 1–PND 15 (W)	0, 3, 30	DX, RX	Repro	30			
					Develop	3	30		Decreased testes weight and increased serum LH and FSH at PND 15
Carbone et al. 2012									
80	Rat (Wistar) 5 F	30 days PNDs 1–21 (via dam) PNDs 22–30 (W)	0, 30	DX	Develop	30			
Carbone et al. 2013									
81	Rat (Wistar) 5 F	45 days PNDs 1–21 (via dam) PNDs 22–45 (W)	0, 30	DX	Develop	30F	30 M		Increased anxiety-like behavior in elevated plus maze
Carbone et al. 2013									
82	Rat (Wistar) 5 F	60 days PNDs 1–21 (via dam) PNDs 22–60 (W)	0, 30	DX	Develop	30 F	30 M		Increased anxiety-like behavior in elevated plus maze, decreased serum testosterone, and increased serum LH
Carbone et al. 2013									
83	Rat (Sprague-Dawley) 8–10 F	31 days GD 12–PND 21 (GO)	0, 10, 100, 750	BW, DX	Bd wt	750			
					Develop			10	>10% decrease in body weight at PND 21 at ≥10 mg/kg/day; >10% decrease in birth weight at ≥100 mg/kg/day; increased thickness of alveolar septa and increased interstitial lung tissue proportion at 750 mg/kg/day
Chen et al. 2010									
84	Rat	31 days			Bd wt	100			

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
	(Wistar) 8–16 F	GD 7–PND 16 (GO)	0, 3, 10, 30, 100	BI, BW, DX, RX	Repro	100			
					Develop		3		Mild external genital dysgenesis in males at ≥3 mg/kg/day; decreased LABC muscle weight at ≥10 mg/kg/day; decreased AGD at 100 mg/kg/day
Christiansen et al. 2010									
85	Rat (Wistar) 8–16 F	31 days GD 7–PND 16 (GO)	0, 10, 30, 100, 300, 600, 900	BI, BW, DX, RX	Bd wt	900			
					Repro	900			
					Develop		10		Decreased AGD, increased nipple retention, decreased adrenal gland and LABC muscle weight at ≥10 mg/kg/day; decreased birth weight, mild external genital dysgenesis, decreased reproductive organ weights, and Leydig cell hyperplasia at ≥300 mg/kg/day
Christiansen et al. 2010									
86	Rat (Wistar) 8–10 M	4 weeks (G)	0, 1,000, 5,000, 10,000	LE, CS, BW, FI, WI, OW, HP, NX, RX	Death			10,000	2/8 deaths due to emaciation
					Bd wt	1,000	5,000	10,000	9% decrease in terminal body weight at 5,000 mg/kg/day; 32% decrease in terminal body weight at 10,000 mg/kg/day
					Cardio	1,000			
					Hepatic	1,000			
					Renal	1,000			
					Endocr	1,000			
					Immuno	1,000			
					Neuro	1,000			
					Repro	1,000		5,000	Decreased fertility, decreased testicular weight, severe atrophy

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
									of seminiferous tubules, and diffuse Leydig cell hyperplasia
Dalgaard et al. 2000									
87	Rat (Wistar) 10 M	9 weeks (GO)	0, 125, 250, 500, 1,000	CS, BW, FI, WI, OW, HP, NX	Bd wt	1,000			
					Cardio	1,000			
					Hepatic	1,000			
					Renal	1,000			
					Endocr	1,000			
					Immuno	1,000			
					Neuro	1,000			
					Repro	1,000			
Dalgaard et al. 2000									
88	Rat (Wistar) 10–12 F	42 days GD 1–PND 21 (GO)	0, 20, 100, 500	BW, DX, RX	Bd wt	500			
					Repro	100		500	Increased post-implantation loss, decreased litter size
					Develop	20	100	500	Decreased plasma testosterone in adult offspring at ≥100 mg/kg/day; altered sexual behavior, decreased sperm production, and decreased reproductive organ weights at 500 mg/kg/day
Dalsenter et al. 2006									
89	Rat (Wistar) 20 F	4 weeks GD 0–PND 7 (GO)	0, 30, 300, 750	DX	Develop		30		Decreased total T4, increased TSH levels, and altered ultrastructure of thyroid follicular cells at PND 7
Dong et al. 2019									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
90	Rat (Wistar) 20 F	5 weeks GD 0–PND 14 (GO)	0, 30, 300, 750	DX	Develop		30		Decreased total T4, increased TSH levels, and altered ultrastructure of thyroid follicular cells at PND 14
Dong et al. 2019									
91	Rat (Wistar) 20 F	6 weeks GD 0–PND 21 (GO)	0, 30, 300, 750	DX	Develop		30		Altered ultrastructure of thyroid follicular cells at PND 21; decreased T4 and increased TSH at ≥300 mg/kg/day
Dong et al. 2019									
92	Rat (Fischer-344) 5–10 M	28 days (F)	0, 23.8, 51.7, 115, 559, 1,093, 2,496	BW, FI, EA, HP, OW	Bd wt	1,093			35% decrease in body weight and 52% decrease in food consumption at 2,496 mg/kg/day
					Hepatic	1,093	2,496		Increased hepatocyte cytoplasmic eosinophilia Increased liver weight and peroxisome proliferation at ≥115 mg/kg/day[b]
					Repro	1,093	2,496		Decreased testes weight, bilateral testicular atrophy
Exxon Chemical Americas 1990									
93	Rat (Long-Evans) 19–38 M	28 d PNDs 21–48 (GO)	0, 10, 500, 750	DX	Develop		10		Decreased age of PPS, increased seminal vesicle weight, and increased serum testosterone at 10 mg/kg/day; opposite reproductive effects observed at 750 mg/kg/day (biphasic response); 13% decrease in body weight at 750 mg/kg/day
Ge et al. 2007									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
94	Rat (Sprague-Dawley) 15 M, 15 F	17 weeks (F)	M: 0, 142, 737, 1,440 F: 0, 154, 797, 1,414	BC, BW, CS, FI, HE, HP, OW, UR, WI	Bd wt	154	797 F		10 decrease in terminal body weight in females with no significant change in mean food consumption; body weight decreases in males at ≥737 mg/kg/day attributed to decreased food consumption
					Resp	1,440			
					Cardio	1,440			
					Gastro	1,440			
					Hemato	142	737		Decreased PCV and hemoglobin
					Musc/skel	1,440			
					Hepatic	1,440			Increased liver weight at ≥142 mg/kg/day[b]
					Renal	142	737		Increased relative kidney weight at ≥737 mg/kg/day; mild renal impairment at 1,414 mg/kg
					Endocr	142 M	737 M		Vacuolation of basophils in the pars distalis in the pituitary gland (“castration cells”) in males
						1,414 F			
					Immuno	1,440			
					Neuro	1,440			
					Repro		142 M		Testicular lesions at ≥142 mg/kg/day; decreased absolute and relative testicular weight at ≥747 mg/kg/day
						1,414 F			
					Other noncancer	797 F	1,414 F		Extensive fur loss on head and ventral body surface

Gray et al. 1977

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
95	Rat (Sprague-Dawley) 13–14 F	31–78 days GD 8–PND 17 (via dam) PNDs 18–64 (direct) (GO)	0, 11, 33, 100, 300	BW, DX, RX	Bd wt	300			
					Repro	300			
					Develop			11	Reproductive tract malformations and nipple retention in adult male offspring at ≥11 mg/kg/day; decreased AGD at PND 2 and decreased reproductive organ weights and sperm count in adult offspring at ≥100 mg/kg/day
Gray et al. 2009									
96	Rat (Sprague-Dawley) 15 M	9 weeks (GO) PNDs 42–105	0, 0.03, 0.1, 0.3, 1	BW, OW, RX	Bd wt	1			
					Repro	0.03	0.1		167% increase in percent sperm with bent tails
Hsu et al. 2016									
97	Rat (Sprague-Dawley) 9–10 M, 9–10 F	13 weeks PNDs 6–96 (direct) (GO)	0, 0.3, 3, 30, 150	CS, BW, FI, BC, BI, HE, OW, GN, HP, DX	Bd wt	150			
					Hemato	150			
					Hepatic	150			Increased absolute and relative liver weight at 150 mg/kg/day[b]
					Renal	30	150		≥10% increase in relative kidney weight
					Endocr	30 M	150 M		Increased thyroid cell proliferation in females at ≥30 mg/kg/day and males at 150 mg/kg/day; thyroid hyperplasia and hypertrophy in both sexes at 150 mg/kg/day
						3 F	30 F		
					Repro	150 M			16–17% decreased in absolute and relative left ovary weight
						3 F	30 F		
					Develop	150			
Kim et al. 2018c, 2018d									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
98	Rat (Sprague-Dawley) 8 F	36 days GD 6–PND 20 (GO)	0, 25, 100, 400	BW, DX	Bd wt	400			
					Repro	400			
					Develop	400			
Kobayashi et al. 2006									
99	Rat (Long-Evans) 8 M	21 days (GO)	0, 10, 750	BC, RX	Bd wt	750			
					Repro		10		Increased serum LH, increased number and proliferation of Leydig cell precursors following elimination of mature Leydig cells using EDS
Li et al. 2012a									
100	Rat (Long-Evans) 8 M	35 days (GO)	0, 10, 750	BC, RX	Bd wt	750			
					Repro		10		Decreased serum testosterone, increased number of Leydig cell precursors following elimination of mature Leydig cells using EDS
Li et al. 2012a									
101	Rat (Long-Evans) 2–6 F	19 days GDs 2–20 (GO)	0, 10, 100, 750	BW, DX, RX	Bd wt	750			
					Repro	750			
					Develop		10		PND 1 males: altered distribution of Leydig cells, decreased testicular testosterone; reduced testes weight and Leydig cell number/volume at ≥100 mg/kg/day; decreased AGD at 750 mg/kg/day
Lin et al. 2008									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
102	Rat (Long-Evans) 11–13 F	31 days GD 12.5–PND 21.5 (GO)	0, 10, 750	BW, DX, RX	Bd wt	750			
					Repro	750			
					Develop		10		Birth (males): altered Leydig cell clustering in males PND 21 males: decreased serum testosterone at ≥10 mg/kg/day; decreased AGD at 750 mg/kg/day
Lin et al. 2009									
103	Rat (Wistar) 10–12 F	42 days GD 0–PND 21 (GO)	0, 1.25, 6.25	BC, BW, RX	Endocr	6.25			
					Repro	6.25			
					Develop			1.25	≥10% decrease in body weight; decreased adipose tissue; pancreatic damage with impaired glucose homeostasis in adult offspring
Lin et al. 2011									
104	Rat (Wistar) 12 F	4 weeks PNDs 22–49 (direct) (GO)	0, 250, 500, 1,000	DX	Develop		250		Increased serum GH at ≥250 mg/kg/day; longer estrous cycle, increased hypothalamic GH, increased serum progesterone, and decreased serum FSH, LH, and testosterone at ≥500 mg/kg/day; accelerated vaginal opening at 1,000 mg/kg/day
Liu et al. 2018a									
105	Rat (Sprague-Dawley) 6 M	30 days (GO)	0, 500	BW, FI, NX	Bd wt	500			
					Neuro		500		Increased anxiety
Liu et al. 2018b									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
106	Rat (Wistar) 3 F	21 days PNDs 1–21 (GO)	0, 1, 10, 100	DX	Develop		1		Altered glucose homeostasis in PND 60 offspring
Mangala Priya et al. 2014									
107	Rat (Wistar) 20 M, 20 F	9 months (F)	0, 50, 200, 1,000	BI, BW, FI, HP, OW	Bd wt	200	1,000		12–15% decreased body weight gain; no change in food consumption
					Hepatic		50		Morphological changes in bile ducts; increased liver weight, hepatocellular hypertrophy, enzyme induction
Mitchell et al. 1985									
108	Rat (Fischer-344) 10 M, 10 F	13 weeks (F)	M: 0, 62.7, 261.2, 850.1, 1,724.0 F: 0, 72.5, 301.8, 918.4, 1,857.6	CS, BW, CS, FI, HE, HP, OP, OW, UR	Bd wt	301.8 F	918.4 F		7% decrease in terminal body weight (22% decrease in body weight gain) with no significant changes in food consumption at 918.4 mg/kg/day; 20% decrease in terminal body weight (55% decrease in body weight gain) and 8% decrease in food consumption at 1.857.6 mg/kg/day
						850.1 M	1,724 M		17% decrease in terminal body weight (38% decrease in body weight gain) with no significant changes in food consumption
					Resp	1,857.6			
					Cardio	1,857.6			
					Gastro	1,857.6			
					Hemato	261.2 M	850.1 M		Decreased RBCs, hemoglobin, and hematocrit and increased platelets
						918.4 F	1,857.6 F		Decreased hemoglobin, hematocrit, segmented

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
									neutrophils, and myeloid/erythroid ratio
					Musc/skel	1,857.6			
					Hepatic	1,724			Increased liver weight at ≥62.7 mg/kg/day; hepatocellular enlargement at ≥261.2 mg/kg/day[b]
					Renal	62.7 M	261.2 M		Increased BUN at ≥261.2 mg/kg/day; increased kidney weight at ≥850.1 mg/kg/day
						301.8 F	918.4 F		Increased kidney weight and BUN
					Ocular	1,857.6			
					Endocr	261.2	850.1		Increased serum glucose at ≥850.1 mg/kg/day; vacuolation in the zona glomerulosa in adrenal gland and increased “castration cells” in pituitary gland (males only) at high dose
					Immuno	1,857.6			
					Neuro	1,857.6			
					Repro	850.1 M	1,724 M		Decreased testis weight, bilateral atrophy and focal mineralization in the testes, and aspermia in the epididymides
						918.4 F	1,857.6 F		Decreased uterus weight
Myers 1992b									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
109	Rat (Sprague-Dawley) 12–14 F	5 weeks GD 8–PND 21 (GO)	0, 30, 300	CS, BC, BW, OW, RX, DX	Bd wt	300			
					Cardio	30	300		13% decrease in relative heart weight
					Hepatic	300			
					Repro	300			
					Develop	30	300		Males: 10% decrease in AGI and increased multinucleated gonocytes (PND 3); increased incidences of hemorrhagic testes (PND 8); females: delayed vaginal opening
Nardelli et al. 2017									
110	Rat (Sprague-Dawley) 7–8 M	22 days PNDs 23–44 (GO)	0, 100, 300, 900	DX	Develop		100		Decreased Cowper's gland and adrenal weight at ≥100; delayed PPS, increased LH, decreased testicular testosterone production, and decreased weight of male reproductive organs at ≥300 mg/kg/day
Noriega et al. 2009									
111	Rat (Sprague-Dawley) 6 M	35 days PNDs 23–57 (GO)	0, 10, 100, 300, 900	DX	Develop	10	100	900	Decreased prostate weight at ≥100 mg/kg/day; decreased male reproductive organ weights and hypospermia/aspermia at ≥300 mg/kg/day; delayed PPS, decreased serum LH, and testicular/epididymal degeneration at 900 mg/kg/day
Noriega et al. 2009									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
112	Rat (Long-Evans) 6 M	35 days PNDs 23–57 (GO)	0, 10, 100, 300, 900	DX	Develop	10	100	900	Decreased Cowper's gland weight at ≥100 mg/kg/day; decreased male reproductive organ weights at ≥300 mg/kg/day; delayed PPS, hypospermia/ aspermia, testicular/epididymal degeneration, and decreased adrenal gland weight at 900 mg/kg/day
Noriega et al. 2009									
113	Rat (Sprague-Dawley) 8 M	42 days PNDs 23–64 (GO)	0, 100, 300, 900	DX	Develop		100		Decreased Cowper's gland weight at ≥100 mg/kg/day; increased LH, decreased testicular testosterone production, delayed PPS, and decreased weight of male reproductive organs at ≥300 mg/kg/day
Noriega et al. 2009									
114	Rat (Sprague-Dawley) 4 M	76 days PNDs 23–98 (GO)	0, 10, 100, 300, 900	DX	Develop	300		900	Delayed PPS, testicular degeneration, 70% decrease in sperm count, decreased testes and epididymides weight, increased serum LH
Noriega et al. 2009									
115	Rat (Long-Evans) 4 M	76 days PNDs 23–98 (GO)	0, 10, 100, 300, 900	DX	Develop	100	300		Delayed PPS at ≥300 mg/kg/day; increased kidney weight at 900 mg/kg/day
Noriega et al. 2009									
116	Rat (Fischer-344) 10 M, 10 F	13 weeks (F)		CS, HP	Resp Cardio Gastro Musc/skel	3,000 3,000 3,000 3,000			

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
			M: 0, 150, 300, 620, 1,300, 2,600 F: 0, 180, 340, 700, 1,400, 3,000		Hepatic	3,000			
					Renal	3,000			
					Endocr	3,000			
					Immuno	3,000			
					Neuro	3,000			
					Repro	620 M 3,000 F		1,300 M	Testicular atrophy
NTP 1982									
117	Rat (Wistar) 6 M	15 days (F)	0, 2000	LE	Death			2,000	50% mortality after 3 weeks with subsequent 100% mortality
Parmar et al. 1987									
118	Rat (Wistar) 6 M	30 days PNDs 25–54 (GO)	0, 50, 100, 250, 500	DX	Develop		50	250	Decreased absolute testes weight at ≥50 mg/kg/day, decreased relative testes weight at ≥100 mg/kg/day, testicular germ cell damage at ≥250 mg/kg/day
Parmar et al. 1995									
119	Rat (Wistar) 3 F	3 weeks PNDs 1–21 (GO)	0, 1, 10, 100	DX	Develop		1	10	LOAEL: 5–9% decrease in body weight from PND 9 to 22 Serious LOAEL: ≥10% decrease body weight from PNDs 9–12 at ≥10 mg/kg/day; 10% increase in fasting blood glucose levels observed at 100 mg/kg/day
Parsanathan et al. 2019									
120	Rat (Sprague-Dawley) 12 F	16 days (GO)	0, 37.5, 75, 150, 300	BI, BW, HP, OW, IX	Bd wt	300			
					Immuno	300			
Piepenbrink et al. 2005									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
121	Rat (Sprague-Dawley) 12–13 F	16 days GDs 6–21 (GO)	0, 37.5, 75, 150, 300	DX, RX	Repro	300			
					Develop		37.5		Increased AGD
Piepenbrink et al. 2005									
122	Rat (Sprague-Dawley) 10 M, 10 F	13 weeks (F)	M: 0, 0.4, 3.7, 37.6, 375.2 F: 0, 0.4, 4.2, 42.2, 419.3	BC, BI, BW, CS, EA, FI, GN, HE, HP, OW	Bd wt	419.3			
					Resp	419.3			
					Cardio	419.3			
					Gastro	419.3			
					Hemato	37.6 M	375.2 M		Decreased RBCs and hemoglobin
						419.3 F			
					Musc/skel	419.3			
					Hepatic	37.6	375.2		Decreased serum cholesterol; increased liver weight, mild hypertrophy, and peroxisomal proliferation
					Renal	37.6	375.2		Increased kidney weight
					Dermal	419.3			
					Ocular	419.3			
					Endocr	419.3			
					Neuro	419.3			
					Repro	3.7 M	37.6 M	375.2 M	Mild vacuolation of Sertoli cells at ≥37.6 mg/kg/day; testicular atrophy and lack of spermatogenesis at 375.2 mg/kg/day
						419.3 F			
Poon et al. 1997									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
123	Rat (Wistar) 6 F	6 weeks GD 9–PND 21 (GO)	0, 10, 100	DX	Develop		10		13% decrease in body weight; altered glucose homeostasis; decreased serum testosterone and estradiol; increased serum ALT (116%), AST (200%), ALP (34%), urea (117%), and creatinine (50%)
Rajagopal et al. 2019a [Endpoints were assessed in male PND 80 offspring.]									
124	Rat (Wistar) 6 M	30 days (GO)	0, 10, 100	BC, BI	Other noncancer		10		Altered glucose metabolism/ homeostasis
Rajesh et al. 2013									
125	Rat (Sprague-Dawley) 4 M, 4 F	5 months (F)	0, 100, 300	NX	Neuro		100		Impaired spatial learning
Ran et al. 2019									
126	Rat (Wistar) 10 M, 10 F	~19 weeks (2-generation) (F)	0, 130, 380, 1,040	BW, CS, DX, FI, HP, RX, OW	Death			1,040	3/9 F1 males and 2/9 F1 females died
					Bd wt	380 F			Decreased F0 and F1 body weight and food consumption at 1,040 mg/kg/day
						1,040 M			
					Hepatic	1,040			Increased liver weights in adult females at ≥130 mg/kg/day and adult males at ≥380 mg/kg/day[b]
					Renal	1,040			
					Endocr	1,040			
					Repro	380	1,040		Observed in one or both generations: decreased fertility, pups/dam, post-implantation loss, decreased reproductive organ weight, testicular lesions

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Develop	130	380	1,040	Decreased spermatocytes in F1 males at ≥380 mg/kg/day; decreased F1 postnatal survival, decreased pup weight, increased nipple retention and decreased AGD in males, and delayed sexual maturation at 1,040 mg/kg/day
Schilling et al. 1999									
127	Rat (Wistar) 25 M, 25 F	19 weeks (2-generation) ~10 weeks premating–PND 21 (F)	0, 113, 340, 1,088	LE, CS, BW, FI, OW, HP, RX, NX	Death			1,088 F	6/25 deaths in F1 adult females
					Bd wt	340			Decreased body weight and food consumption in F0 females and adult F1 males and females at 1,088 mg/kg/day
					Hepatic	113	340		F1 adults: hepatocellular eosinophilia, increased liver weight at ≥340 mg/kg/day; focal bile duct proliferation and altered hepatic foci at 1,088 mg/kg/day
					Renal		113		Increased relative kidney weight in F0 and F1 adults at ≥113 mg/kg/day; renal tubule dilation and renal pelvis calcification in F1 adults at 1,088 mg/kg/day
					Endocr	1,088			
					Immuno	1,088			
					Neuro	1,088			
					Repro		113 M	1,088 M	Focal tubular atrophy in testis in F1 males at ≥113 mg/kg/day and F0 males at 1,088 mg/kg/day; aspermia and decreased fertility in F1 at 1,088 mg/kg/day

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
						340 F	1,088 F		Increased post-implantation loss in F0 females; decreased growing ovarian follicles and corpora lutea in F0 and F1 females
					Develop	113		340	Decreased pup survival, decreased pup weight gain, decreased AGD/AGI, and increased nipple retention at ≥340 mg/kg/day; delayed F1 sexual maturation at 1,088 mg/kg/day; increased pup liver weight at ≥113 mg/kg/day[b]
Schilling et al. 2001									
128	Rat (Wistar) 5 M	90 days (F)	0, 200, 400, 900, 1,900	BC, BW, HP	Cardio	1,900			
					Hemato	1,900			
					Hepatic	1,900			
					Renal	1,900			
					Immuno	1,900			
					Repro	400		900	Tubular atrophy and degeneration
Shaffer et al. 1945									
129	Rat (Wistar) 12 F	4 weeks PNDs 15–43 (GO)	0, 0.2, 1, 5	CS, DX	Develop	0.2	1		20-30% increase in serum IGF-1 and serum and hypothalamic GnRH at 1 mg/kg/day; increased hypothalamic IGF-1, accelerated vaginal opening, and clinical signs of toxicity (lassitude, anorexia, hair loss and yellowing) at 5 mg/kg/day
Shao et al. 2019									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
130	Rat (Wistar) 10 M, 10F	28 days (GO)	0, 5, 50, 500	BW, BC, BI	Bd wt		5		10% increase in body weight
					Endocr	50	500		Increased serum total T3 and T4, increased hypothalamic thyrotropin-releasing hormone
Sun et al. 2018									
131	Rat (Wistar) 5 F	3 weeks PNDs 1–21 (GO)	0, 7.5, 75	CS, BW, FI, DX	Bd wt	75			
					Develop		7.5		Effects in offspring at PNDs 90–92: Impaired glucose homeostasis and decreased serum triglycerides at ≥7.5 mg/kg/day; decreased serum at 75 mg/kg/day
Venturelli et al. 2015									
132	Rat (Wistar) 15 M	30 days PNDs 22–52 (GO)	0, 7.5, 75	DX	Develop	7.5	75		30% increase in fasting serum glucose
Venturelli et al. 2015									
133	Rat (Wistar) 7–8 F	4 weeks GD 13–PND 21 (GO)	0, 7, 70, 700	BW, OW, RX, DX	Bd wt	700			
					Repro	700			
					Develop	7	70	700	LOAEL: Decreased insulin secretion in pancreatic islet cells in males; delayed vaginal opening in females Serious LOAEL: Hypospadias; additional effects at this dose included >10% increase in body weight postweaning in both sexes, reduced AGI in males, delayed preputial separation, altered glucose homeostasis, increased serum cholesterol in males

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Other noncancer	700			
Venturelli et al. 2019									
134	Rat (Sprague-Dawley) 4 M	15 days PNDs 21–35 (GO)	0, 10, 100, 500	DX	Develop		10		Decreased serum testosterone, decreased reproductive organ weights, degeneration of the Leydig cells, and “disorders of germ cells” at ≥10 mg/kg/day; dilation of tubular lumen and germ cell stratification at ≥100 mg/kg/day
Vo et al. 2009b									
135	Rat (Sprague-Dawley) 12 F	5 weeks GD 7–PND 21 (GO)	0, 0.01, 0.1, 1	DX	Develop	0.01	0.1		Decrease in absolute (52%) and relative (46%) epididymal weight in adult offspring
Wang et al. 2017a [Male offspring sacrificed on PND 196.]									
136	Rat (Wistar) 6–7 F	6 weeks GD 0–PND 21 (G)	0, 30, 300	DX	Develop		30		Enhanced immune response to OVA challenge in sensitized offspring
Wang et al. 2018 [OVA-sensitized offspring evaluated on PNDs 14, 21, and 28.]									
137	Rat (Sprague-Dawley) 6 M	30 days (W)	0, 300, 1,000, 3,000	BW, BC, OW, HP	Bd Wt		300		20% increase in body weight gain
					Gastro	3,000			
					Hepatic		300		Decreased serum cholesterol at ≥300 mg/kg/day; mild steatosis at ≥1,000 mg/kg/day; 21% increase in relative liver weight and increased serum ALT, ALP, and AST at 3,000 mg/kg/day
					Immuno		300		Increased IL-12 and TNF-α at ≥300 mg/kg/day; increased IFN-γ and IL-2 at 3,000 mg/kg/day

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Repro		300		Mild localized necrotic degeneration of seminiferous tubules and interstitial edema at ≥300 mg/kg/day; 60% decrease in serum testosterone at 3,000 mg/kg/day
Wang et al. 2020									
138	Rat (Wistar) 6 M	30 days (W)	0, 300, 1,000, 3,000	BW, BC, OW, HP	Bd Wt	3,000			
					Gastro	3,000			
					Hepatic	300	1,000		Slight centrilobular steatosis at ≥1,000 mg/kg/day; decreased serum cholesterol at 3,000 mg/kg/day
					Immuno	3,000			
					Repro	3,000			
Wang et al. 2020									
139	Rat (Wistar) 10 F	42 days GD 0–PND 21 (GO)	0, 0.25, 6.25	BW, DX, RX	Repro	6.25			
					Develop		0.25	6.25	Kidney lesions and impaired renal development and at PNWs 0–33 at ≥0.25 mg/kg/day; >10% decrease in body weight through adulthood, elevated blood pressure, and increased kidney weight at 6.25 mg/kg/day
Wei et al. 2012									
140	Rat (Wistar) 10 M, 10 F	28 days (GO)	0, 5, 50, 500	BW, FI, WI BC, OW	Bd wt	500			
					Hepatic	500			Increased relative liver weight at 500 mg/kg/day[b]
					Other noncancer	5	50		Altered glucose homeostasis; increased serum leptin
Xu et al. 2018									
141	Rat (Wistar)	30 days (G)	0, 0.7, 70	HP, BI, IX	Immuno		0.7		Enhanced immune response to OVA challenge in sensitized

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
	8 M								animals; non-sensitized animals showed mild increases in immune response at 70 mg/kg/day (not tested at 0.7 mg/kg/day)
Yang et al. 2008									
142	Rat (Sprague-Dawley) 6 M	30 days (GO)	0, 250, 500, 750	LE, CS, BW, BC, OW, HP	Bd wt	750			
					Hepatic	250	500		Vacuolation, hepatic sinusoidal dilation, and reduction in hepatocyte number at ≥500 mg/kg/day; Increased liver weight and hepatocellular hypertrophy at ≥250 mg/kg/day[b]
					Endocr		250		Increased number of thyroid follicular epithelial cells at ≥250 mg/kg/day; decreased serum TT4, FT4, and TT3 at ≥500 mg/kg/day; decreased serum FT3 and TRH and follicular cavity diameter and altered thyroid ultrastructure at 750 mg/kg/day
Ye et al. 2017									
143	Rat (Sprague-Dawley) 10 M	15 weeks (GO)	0, 0.05, 5, 500	BW, BC, OW, HP	Bd wt	500			
					Hepatic		0.05		Vacuolar degeneration and inflammatory infiltration at ≥0.05 mg/kg/day; 26% increase in relative liver weight and 145% increase in serum ALP at ≥5 mg/kg/day; increased serum ALT (100%) and AST (70%) and central necrosis at 500 mg/kg/day
					Other noncancer	0.05	5		Altered glucose homeostasis
Zhang et al. 2017									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
144	Rat (Sprague-Dawley) 8 M	31 days (GO)	0, 500	CS, BW, FI, BC, OW, HP	Bd wt	500			
					Endocr		500		Decreased free T4 and TSH; microscopic and ultrastructural changes in thyroid follicular cells
Zhang et al. 2018b									
145	Rat (Wistar) 10 M, 10 F	8 weeks (GO)	0, 5, 50, 500	BW, BC, HP	Bd wt	500			
					Hepatic		5		Disordered hepatocyte cords and vacuolar degeneration at ≥5 mg/kg/day; increased serum cholesterol and HDL at 500 mg/kg/day
					Other noncancer		5		Increased volume of adipocytes at ≥5 mg/kg/day; increased number of adipocytes at 500 mg/kg/day
Zhang et al. 2019, 2020c									
146	Rat (Wistar) 10 M, 10 F	8 weeks (GO)	0, 5, 50, 500	BW, BC, HP	Bd wt	50	500		>10% increase in body weight
					Hepatic	50	500		30% in increase cholesterol, 95% increase in HDL, 26% increase in LDL
					Other noncancer	5	50		Irregular adipocytes and macrophage infiltration in adipose tissue, increased serum leptin and decreased adiponectin at ≥50 mg/kg/day; increased number and volume of adipocytes at 500 mg/kg/day
Zhou et al. 2019									
147	Mouse (C57BL/6J x FVB) 6 F	9 weeks 2 weeks pre-mating to PND 21 (F)	0, 0.003, 0.03, 0.3, 3.3, 10, 33, 100	LE, CS, BW, FI, RX, DX	Bd wt	100			
					Repro	100			
					Develop	100			

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Bastos Sales et al. 2018 [Some endpoints (locomotor activity, object recognition, and glucose homeostasis at weeks 27–40) were only assessed at 33 mg/kg/day.]									
148	Mouse (CD-1) 10 B	10 weeks 2 weeks premating – PND 21 (W)	0, 0.034, 0.34	BW, OW, HP, RX	Repro	0.34			
Cha et al. 2018									
149	Mouse (CD-1) 12 M	8 weeks (GO)	0, 1, 10, 100	BW, OW, HP	Bd wt	100			
					Musc/ Skel	1	10		25% decrease in trabecular bone mineral density, 17% decrease in bone volume fraction, reduced osteoblastogenesis and mineralization of bone marrow stromal cells; reduced trabecular bone thickness and cell number at 100 mg/kg/day
Chiu et al. 2018c									
150	Mouse (C57/BL6) 8 M	6 weeks (G)	0, 0.1, 1, 10	BC, BI, HP, OF	Cardio		0.1		13% increase in systolic blood pressure, thickening of interventricular septum and ventricular wall; increased heart rate at ≥1 mg/kg/day
Deng et al. 2019									
151	Mouse (ICR) 10 M	3 weeks (IN)	0, 0.18, 1.8, 18, 180	CS, BW, BC, BI, OW, OF	Bd wt	180			
					Cardio	18	180		10% increased heart rate, 29% increased mean blood pressure
					Hepatic	0.18	1.8		Increased total cholesterol at 1.8 mg/kg/day; decreased HDL cholesterol at ≥18 mg/kg/day; increased triglycerides, LDL cholesterol, ALT, and ALP at 180 mg/kg/day

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Other noncancer	18	180		60% increase in blood glucose
Ding et al. 2019									
152	Mouse (ICR) 6 F	28 days 7 days premating – PND 0 (G)	0, 0.2	DX	Develop		0.2		15% increase in body weight, increased white adipose tissue, and metabolic syndrome (reduced energy expenditure, abnormal glucose metabolism, and altered lipid profile) in male offspring
Fan et al. 2020 [Offspring were evaluated at PNW 12.]									
153	Mouse (ICR) 10 M	3 weeks (GO)	0, 0.18, 1.8, 18, 180	BW, BI, OW, NX	Bd wt	180			
					Neuro		0.18		Impaired learning and memory and reduced swim speed at ≥0.18 mg/kg/day; decreased anxiety at ≥1.8 mg/kg/day; decreased locomotor activity at 18 mg/kg/day
					Repro	18	180		15% decrease in relative testes weight
Feng et al. 2020									
154	Mouse (C57Bl/6J) 6–7 F	19 days GD1–19 (GO)	0, 0.05, 500	FI, RX, DX	Repro	0.05		500	100% litter loss
					Develop		0.05		Metabolic syndrome in PNW 9 offspring: increases in serum leptin (11–13%) and insulin (22-26%), fasting glucose levels (16%), and visceral fat weight (24–37%)
Gu et al. 2016									
155	Mouse (BALB/c) 8 M	52 days (G)	0, 0.03, 0.3, 3	BC, HP, IX	Immuno		0.03		Enhanced immune response to OVA challenge in sensitized animals (80% increase in serum total IgE); enhanced responses in

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
									non-sensitized animals at 3 mg/kg/day
Guo et al. 2012									
156	Mouse (BALB/c) 4 M, 4 F	28 days (GO)	0, 0.03, 0.3, 3	IX	Immuno		0.03		Enhanced humoral immune response to OVA challenge in sensitized animals (45–75% increase in OVA-specific IgE and IgG)
Han et al. 2014a									
157	Mouse (CD-1) 8 F	30 days (GO)	0, 0.02, 0.2, 20, 200	BW, OW, RX	Bd wt	200			
					Repro	20	200		Increased percentage of days spent in estrus
Hannon et al. 2014									
158	Mouse (A/J) 10 M	4 weeks (F)	0, 12.3, 125	BW, FI, WI, HP	Bd wt	125			
					Repro		12.3		Sertoli cell vacuolation and germ cell sloughing in seminiferous tubules
Kitaoka et al. 2013									
159	Mouse (Cr1:CD-1) 20 M, 20 F	18 weeks (F)	0, 13, 130, 390	BW, BC, OW, HP, DX, RX	Bd wt	390			
					Repro	13	130	390	Decreased fertility and live pups at ≥130 mg/kg/day; male and female infertility, 50% decrease in serum testosterone, and damage to sperm and testes at 390 mg/kg/day
					Develop	13	130		6% decrease in female pup weight
Lamb et al. 1987; Morrissey et al. 1988; NTP 1984 [continuous breeding protocol with crossover mating]									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
160	Mouse (C57BL/6) 17 M	35 days (G)	0, 1, 10, 100, 300	BW, BC, BI, OW, HP	Bd Wt	300			
					Cardio	1	10		≥10% increase in relative heart weight at ≥1 mg/kg/day; increased lipid droplets in cardiac papillary muscle cells at ≥100 mg/kg/day
					Hepatic		1		Increased serum ALT and triglycerides (≥1 mg/kg/day), cholinesterase (≥10 mg/kg/day), and cholesterol at (≥100 mg/kg/day)
					Renal	100	300		43% increase in serum creatinine
					Endocr	10	100		20% increase in serum T4
					Other noncancer	100	300		68% increase in blood glucose
Li et al. 2018									
161	Mouse (B6C3F1) 10 M, 10 F	28 days (F)	M: 0, 245, 1,209, 2,579, 6,922 F: 0, 270, 1,427, 2,888, 7,899	BC, BW, LE, HE, HP, OW	Death			6,922 M	4/10 males died
								7,899 F	3/10 females died
					Bd wt	2,579 M	6,922 M		35% decrease in body weight and 18–20% decrease in food consumption during weeks 1–2 only
						2,888 F		7,899 F	39% decrease in body weight; no change in food consumption
					Resp	7,899			
					Cardio	7,899			
					Gastro	7,899			
					Hemato	245 M	1,209 M		Decreased hemoglobin and hematocrit in males
						1,427 F	2,888 F		Decreased hemoglobin and hematocrit in females

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Hepatic	245	1,209		Slight to moderate focal coagulative necrosis and increased liver weight at ≥1,209 mg/kg/day; increased hepatocellular hypertrophy at ≥2,579 mg/kg/day
					Renal	1,427 F	2,888 F		Tubular necrosis, dilation, and regeneration in females
						2,579 M	6,922 M		Tubular necrosis, dilation, and regeneration in males
					Endocr	7,899			
					Immuno	2,579	6,922		Thymic atrophy
					Neuro	2,579		6,922	Hunched posture in 4/10 males and 10/10 females; hypoactivity in 2/10 females and tremor in 1/10 females
					Repro	1,209 M 7,899 F	2,579 M		Decreased testes weight at ≥2,579 mg/kg/day; testicular atrophy and decreased spermatogenesis at 6,922 mg/kg/day
Myers 1992a									
162	Mouse (B6C3F1) 10 M, 10 F	13 weeks (F)	M: 0, 150, 300, 600, 1,200, 2,500 F: 0, 170, 330, 640, 1,300, 2,600	CS, HP	Resp	2,600			
					Cardio	2,600			
					Gastro	2,600			
					Musc/skel	2,600			
					Hepatic	2,600			
					Renal	2,600			
					Endocr	2,600			
					Immuno	2,600			
					Neuro	2,600			
					Repro	2,600			

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
NTP 1982									
163	Mouse (CD-1) 28–29 F	18 days GDs 0–17 (F)	0, 19, 48, 95	BW, FI, DX, RX	Bd wt	95			
					Repro	48	95		19% decrease in live pups/litter
					Develop	48		95	11% decrease in postnatal viability from PND 1 to 4
NTP 1988									
164	Mouse (CD-1) 6 F	30 days (IN)	0, 0.02, 0.2, 2	RX	Repro	0.02	0.2		Increased unfertilized oocyte rate and percent of zygotes with fragmentation, arrested zygote development, and decreased preimplantation embryos
Parra-Forero et al. 2019 [Females mated to untreated males; uterine contents evaluated at 24, 48, 74, 84, or 96 hours post-mating.]									
165	Mouse (CD-1) 7–10 F	42 days GD 0–PND 21 (F)	0, 0.05, 5, 500	OW, RX, DX	Repro	5		500	Complete litter loss in 9/10 dams
					Develop			0.05	>20% decrease body weight, decreased adipose tissue, decrease in sperm count and viability, decrease in seminal vesicle weight, increase in ovary weight
Pocar et al. 2012									
166	Mouse (NC/Nga) 12 M	4 weeks 1 day/week (GO)	0, 0.0475, 0.095, 19	BC, CS, HP, IX	Immuno	19			
Sadakane et al. 2014 [mite-sensitized mice]									
167	Mouse (C57BL/6) 6 M	20 days (F)	0, 180, 360	BW, OW, IX	Bd wt	360			
					Immuno	360			
Sasaki et al. 2003									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
168	Mouse (C3H/N) 15 F	8 weeks 7 weeks premating GD1 (F)	0, 0.05, 5, 500	BW, CS, FI, RX	Bd wt		0.05		~18% increase in body weight
					Repro	500			
					Other noncancer		0.05		Increased visceral adipose tissue and adipocyte hypertrophy at ≥0.05 mg/kg/day; increased serum leptin at 500 mg/kg/day
Schmidt et al. 2012									
169	Mouse (C3H/N) 15 F	8 weeks 7 weeks premating–GD 1 (F)	0, 0.05, 5, 500	BW, CS, FI, RX, DX	Repro	500			
					Develop			0.05	>20% increase in offspring body weight at PND 21, increased visceral adipose tissue
					Other noncancer		0.05		Increased visceral adipose tissue and adipocyte hypertrophy at ≥0.05 mg/kg/day; increased serum leptin at 500 mg/kg/day
Schmidt et al. 2012									
170	Mouse (ICR) 12–17 F	18 days GDs 0–17 (GO)	0, 50, 200	LE, BW, FI, RX, DX	Bd wt	200			
					Repro	200			
					Develop			50	≥10% decrease in fetal weight, 4% decrease in crown-rump length
Shen et al. 2017									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
171	Mouse (ICR) 7–12 F	18 days GDs 1–18 (F)	0, 85, 170, 341, 683, 1,707	BW, FI, RX, DX	Bd wt	170		341	26% decrease in maternal weight at GD 18; no change in food consumption
					Repro	170		341	62.8% increase in resorptions and fetal mortality (combined); complete litter loss at ≥683 mg/kg/day
					Develop	170		341	14–21% decrease in GD 18 fetal weight; 25.8 % increase in number of malformed fetuses
Shiota et al. 1980; Shiota and Nishimura 1982									
172	Mouse (CD-1) 10 M, 10 F	17 weeks 4 weeks premating–PNW 9 (F)	0, 20.62, 60.42, 180.77	BW, FI, RX, DX, NX	Bd wt	180.77			
					Neuro	180.77			
					Repro	180.77			
					Develop		20.62 F	180.77 F	Delayed surface righting reflex on PNDs 4 and 7 at ≥20.62 mg/kg/day in females; decreased female survival during lactation at 180.77 mg/kg/day
						60.42 M	180.77 M		Delayed surface righting reflex on PNDs 4 and 7
Tanaka 2002 [Reported doses are TWAs across sex and generation.]									
173	Mouse (ICR) 5–6 F	15 days GDs 8–17 (dams) and PNDs 3–7 (pups) (GO)	0, 1	DX	Develop			1	>10% decrease in pup weight at PNW 2; 6–9% decrease in pup weight at PNWs 4–6, increased relative brain weight at PNWs 2 and 4, and decreased number and activity of dopaminergic neurons
Tanida et al. 2009									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
174	Mouse (C57bl/6J/ BALB/cByJ hybrid) 15 M, 15 F	26 weeks (F)	0, 1,100	BW, CS, FI, HP, OW	Bd wt		1,100		~10% decrease in body weight; no change in food consumption
					Resp		1,100		Increased incidence of eosinophilic bodies in nasal cavities
					Cardio	1,100			
					Gastro	1,100			
					Musc/skel	1,100			
					Hepatic	1,100			Elevated absolute and relative liver weight; liver hypertrophy[b]
					Renal		1,100		Tubular regeneration in both sexes; hydronephrosis in females
					Dermal	1,100			
					Ocular	1,100			
					Endocr	1,100			
					Immuno	1,100			
					Neuro	1,100			
					Repro	1,100 F			
							1,100 M		Decreased testis weight, focal testicular atrophy
Toyosawa et al. 2001									
175	Mouse (CD-1) 24–25 F	17 days GDs 0–17 (F)	0, 44, 91, 191, 292	BW, CS, FI, WI, OW, GN, RX	Bd wt	91	191		30% decrease in maternal weight gain; no change in food consumption
					Neuro	44	91		Maternal lethargy
					Repro	91	191		Increased resorptions and late fetal deaths, decreased live pups/litter
					Develop	44		91	Increased incidence of external, visceral, and skeletal abnormalities at ≥91 mg/kg/day;

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
									decreased fetal weight at ≥191 mg/kg/day
Tyl et al. 1988									
176	Mouse (BALB/c) 6 M	30 days (W)	0, 300, 1,000, 3,000	BW, BC, OW, HP	Bd Wt	3,000			
					Gastro	3,000			
					Hepatic	300	1,000		Increased serum ALP at ≥1,000 mg/kg/day; mild steatosis at 3,000 mg/kg/day
					Immuno	3,000			
					Repro	300	1,000		33% decrease in serum testosterone, slight localized degeneration of germ cells
Wang et al. 2020									
177	Mouse (C57BL/6J) 6 M	30 days (W)	0, 300, 1000, 3000	BW, BC, OW, HP	Bd Wt	3,000			
					Gastro	3,000			
					Hepatic		300		Mild inflammatory cell infiltrates at ≥300 mg/kg/day; 11% increase in relative liver weight at 3,000 mg/kg/day
					Immuno		300		Increased IL-1-α at ≥300 mg/kg/day; increased IL-6 and MCP-1 at 3,000 mg/kg/day
					Repro	1,000	3,000		Slight seminiferous tubule atrophy
Wang et al. 2020									
178	Mouse (Sv/129) 15 M	24 weeks (F)	0, 2,400	LE	Death			2,400	100% mortality between weeks 12 and 16
Ward et al. 1988									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
179	Mouse (C57BL/6) 9 M	45 days (G)	0, 0.1, 1, 10	CS, BW, BC, BI, HP, OF	Cardio		0.1		Increased mean and systolic blood pressure, thickened aortic wall, and hypertrophied and disordered aortic smooth muscle cells at ≥0.1 mg/kg/day; increased diastolic blood pressure at ≥1 mg/kg/day
					Renal	0.1	1		Glomerular damage, increased inflammatory cell infiltration
					Repro	10			
Xie et al. 2019									
180	Mouse (ICR) 7–15 M	28 days (GO)	0, 4, 400	BW, HE BC, OW, HP	Bd Wt	400			
					Hemato	400			
					Hepatic	400			Increased absolute liver weight and hepatocellular hypertrophy at 400 mg/kg/day[b]
					Renal	4	400		10% increase in absolute kidney weight
					Endocr	4	400		145% Increase in absolute adrenal gland weight
					Immuno	400			
					Neuro	400			
					Repro	4	400		24% increase in absolute testis weight; 16% increase in absolute prostate weight
Xu et al. 2019									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
181	Mouse (CD-1) 5 F	20 days GDs 0.5–18.5 (NS)	0, 0.04	BC, DX	Repro		0.04		25% decrease in maternal serum estradiol
					Develop		0.04[d]		Delayed meiotic progression of germ cells in GD 17.5 F1 fetuses (cells in leptotene and zygotene stages increased by 22 and 31%, respectively); accelerated folliculogenesis in F1 and F2 PND 21 offspring (20% increase in secondary follicles)
Zhang et al. 2015									
182	Guinea pig (NS) 4–5 M	15 days (GO)	0, 2,000	LE	Death			2,000	40% mortality
Parmar et al. 1988									
183	Rabbit (NS) NS M	15 days (GO)	0, 2,000	LE	Death			2,000	100% mortality
Parmar et al. 1988									
CHRONIC EXPOSURE									
184	Monkey (Marmoset) 7–8 M, 5–6 F	65 weeks (GO)	0, 100, 500, 2,500	BC, BI, CS, EA, HP, OW	Develop	100 F	500 F		Increased serum estradiol, elevated ovary weights, and enlarged corpora lutea
Tomonari et al. 2006 [exposed from weaning at 3 months until sexual maturation at 18 months]									
185	Rat (Sherman) 32 M, 32 F	2 years (F)	0, 20, 65, 200	BC, BW, HP, OW, RX	Bd wt	200			
					Resp	200			
					Cardio	200			
					Gastro	200			
					Hemato	200			

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Hepatic	200			Increased liver weight at 200 mg/kg/day in F0 and F1 adults
					Renal	65	200		Increased kidney weight in F0 and F1 adults
					Endocr	200			
					Immuno	200			
					Repro	200			
Carpenter et al. 1953 [combined chronic and reproductive study; high-dose F1 animals maintained for 1 year]									
186	Rat (Fischer-344) 20 F	2 years (F)	0, 15, 50, 600	HP, OW	Cancer			600	CEL: hepatocellular carcinoma
Cattley et al. 1987 [The liver was the only organ evaluated.]									
187	Rat (Fischer-344) 50–80 M, 50–80 F	104 weeks (F)	M: 0, 5.8, 29, 147, 789 F: 0, 7.3, 36, 182, 939	BC, BW, CS, FI, HP, OW, UR	Death			147	12% reduction in survival due to mononuclear cell leukemia
					Bd wt	182	789		15% decrease in body weight gain; no changes in food consumption
					Gastro	939			
					Hemato	939			
					Musc/skel	939			
					Hepatic	36 M	147 M		Increased incidence of spongiosis hepatis, increased liver weight, and peroxisome proliferation at ≥147 mg/kg/day; increased cytoplasmic eosinophilia and Kupffer cells at 789 mg/kg/day
						182 F	939 F		Increased cytoplasmic eosinophilia and Kupffer cells
					Renal	36	147		Increased kidney weight at ≥147 mg/kg/day; increased

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
									severity of chronic progressive nephropathy at ≥789 mg/kg/day
					Endocr	147 M	789 M		Vacuolation of basophils in the pars distalis in the pituitary gland ("castration cells") in males
						939 F			
					Immuno	939			
					Neuro	939			
					Repro	5.8 M		29 M	Bilateral testicular aspermatogenesis at ≥29 mg/kg/day; decreased testes weight at 789 mg/kg/day
						939 F			
					Cancer			147 M	CEL: hepatocellular tumors in males at ≥147 mg/kg/day; pancreatic acinar cell adenomas and mononuclear cell leukemia in males at 789 mg/kg/day
								939 F	CEL: hepatocellular tumors in females
David et al. 1999, 2000a									
188	Rat (Sprague-Dawley) 7–18 M	102 weeks (F)	0, 14, 140, 1,400	CS, EA, HP	Repro		14		"Inhibition" of spermatogenesis and general tubule atrophy (magnitude not reported)
Ganning et al. 1991									
189	Rat (Fischer-344) 7–10 M	78 weeks (F)	0, 1,579	BW, HP, OW	Cancer			1,579	CEL: hepatocarcinomas
Hayashi et al. 1994									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
190	Rat (Fischer-344) 50 M, 50 F	2 years (F)	M: 0, 322, 674 F: 0, 394, 774	BW, FI, HP, GN	Bd wt	774			
					Resp	774			
					Cardio	774			
					Gastro	774			
					Musc/skel	774			
					Hepatic		322 M		Increased incidence of clear cell foci in liver
						774 F			
					Renal	774			
					Dermal	774			
					Endocr	322 M	674 M		Anterior pituitary cell hypertrophy
						774 F			
					Immuno	774			
					Neuro	774			
					Repro	322 M		674 M	Severe seminiferous tubular degeneration and testicular atrophy
						774 F			
					Cancer			394 F	CEL: neoplastic liver nodules or hepatocellular carcinoma in females
								674 M	CEL: neoplastic liver nodules or hepatocellular carcinoma in males
Kluwe et al. 1982a, 1982b, 1985; NTP 1982									
191	Rat (Fischer-344) NS M	365 days (F)	0, 930	BW, FI, OW HP, BI	Bd wt	930			
Marsman et al. 1988									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
192	Rat (Wistar) NS	2 years (F)	0, 2,000	HP	Repro			2,000	Testicular atrophy
Price et al. 1987									
193	Rat (Fischer-344) 8–20 M	95 weeks (F)	0, 1,600	BI, HP	Cancer			1,600	CEL: hepatocellular carcinoma
Rao et al. 1987 [Only the liver was examined.]									
194	Rat (Fischer-344) 10–14 M	108 weeks (F)	0, 1,600	BW, HP, OW	Resp	1,600			
					Gastro		1,600		Pseudoductular lesions and altered acinar cell foci in the pancreas
					Renal	1,600			
					Cancer			1,600	CEL: hepatocellular carcinoma, pancreatic islet-cell adenoma
Rao et al. 1990									
195	Rat (Sprague-Dawley) 60–390 M	Lifetime 6 days/week (F)	0, 30, 95, 300	BW, CS, HP, OW	Bd wt	300			
					Resp	300			
					Hepatic	300			
					Endocr	300			
					Immuno	300			
					Neuro	300			
					Repro	95	300		Seminiferous tubule atrophy
					Cancer			300	CEL: Leydig cell tumors
Voss et al. 2005									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
196	Mouse (B6C3F1) 60–70 M, 60–70 F	104 weeks (F)	M: 0, 19.2, 98.5, 292.2, 1,266 F: 0, 23.8, 116.8, 354.2, 1,458	BC, BW, CS, FI, HP, OW, UR	Death			1,266	45% reduced survival due to hepatocellular neoplasia
					Bd wt	292.2 M	1,266 M		9.8% decrease in body weight, no change in food consumption
						1,458 F			
					Gastro	1,458			
					Hemato	1,458			
					Musc/skel	1,458			
					Hepatic	292.2	1,266		Cytoplasmic eosinophilia; increased liver weight, hypertrophy, and peroxisomal proliferation at ≥292.2 mg/kg/day[b]
					Renal	116.8	292.2		Chronic progressive nephropathy
					Endocr	1,458			
					Immuno	1,458			
					Neuro	1,458			
					Repro	98.5 M	292.2 M		Reduced testes weight and hypospermia
						354.2 F	1,458 F		Reduced absolute and relative uterus weight
					Cancer			292.2	CEL: hepatocellular tumors
David et al. 1999, 2000b									
197	Mouse (SV/129) 20–24 M	22 months (F)	0, 9.5, 48.5	BC, BI, BW, HP, OW, UA	Cardio		9.5		Elevated systolic blood pressure (secondary to renal effects)
					Renal		9.5		Mild glomerulonephritis, cell proliferation, proteinuria
Kamijo et al. 2007									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
198	Mouse (B6C3F1) 50 M, 50 F	2 years (F)	M: 0, 672, 1,325 F: 0, 799, 1,821	BW, FI, GN, HP	Bd wt	672 M	1,325 M		10% decrease in terminal body weight, no change in food consumption
								799 F	21% decrease in terminal body weight; no change in food consumption
					Resp	1,821			
					Cardio	1,821			
					Gastro	1,821			
					Musc/skel	1,821			
					Hepatic	1,821			
					Renal	672 M	1,325 M		Chronic inflammation of the kidney
						1,821 F			
					Dermal	1,821			
					Endocr	1,821			
					Immuno	1,821			
					Neuro	1,821			
					Repro	672 M		1,325 M	Seminiferous tubular degeneration
						799 F	1,821 F		Suppurative inflammation in the uterus/endometrium
					Cancer			672	CEL: hepatocellular adenoma or carcinoma

Kluwe et al. 1982a, 1982b, 1985; NTP 1982

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
199	Guinea pig (NS) 46–47 B	1 year (F)	0, 19, 64	BW, OW, HP	Bd wt	64			
					Hepatic	64			Increased female liver weight at 64 mg/kg/day[b]
					Renal	64			
					Immuno	64			
					Repro	64 M			
Carpenter et al. 1953 [Female reproductive organs were not assessed.]									
200	Dog (NS) 1 M, 1 F	1 year 5 days/ week (C)	0, 56.6	BC, BW, HP, OF, OW	Bd wt	56.6			
					Resp	56.6			
					Cardio	56.6			
					Gastro	56.6			
					Hepatic	56.6			
					Renal	56.6			
					Endocr	56.6			
					Immuno	56.6			
					Repro	56.6			
Carpenter et al. 1953									

Table 2-2. Levels of Significant Exposure to DEHP – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
201	Ferret (albino) 7 M	14 months (F)	0, 1,200	BI, BW, EA, OW, HP	Cardio	1,200			
					Hepatic		1,200		Hepatocellular vacuolation, increased liver weight, hypertrophy, enzyme induction
					Endocr	1,200			
					Neuro	1,200			
					Repro			1,200	3/7 with absence of germinal epithelium in seminiferous tubules

Lake et al. 1976

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Hepatic effects associated with hepatomegaly (elevated liver weight, hypertrophy, enzyme induction, and/or peroxisome proliferation) are not considered adverse unless hepatocellular degenerative or necrotic changes or evidence of biliary or other liver cell damage were also present (Hall et al. 2012). The lowest doses associated with hepatomegaly endpoints are noted in the LSE tables even though the dose levels are considered NOAELs.
[c]Used to derive an acute-duration oral minimal risk level (MRL). The LOAEL of 1 mg/kg/day was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for human variability, and 10 for animal to human extrapolation), resulting in an MRL of 0.003 mg/kg/day ($3x10^{-3}$ mg/kg/day).
[d]Used to derive an intermediate-duration oral MRL. The LOAEL of 0.04 mg/kg/day was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for human variability, and 10 for animal to human extrapolation), resulting in an MRL of 0.0001 mg/kg/day ($1x10^{-4}$ mg/kg/day).

AGD = anogenital distance; AGI = anogenital index; ALP = alkaline phosphatase; ALT= alanine aminotransferase; AST = aspartate transaminase; B = both males and females (number per sex not reported); BC = serum (blood) chemistry; Bd Wt or BW = body weight; BI = biochemical changes; BUN = blood urea nitrogen; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; DEHP = di(2-ethylhexyl)phthalate; Develop = developmental; DX = developmental toxicity; EA = enzyme activity; EDS = ethane dimethanesulphonate; Endocr = endocrine; (F) = feed; F = female(s); F0 = parental generation; F1 = first generation; F2 = second generation; FI = food intake; FSH = follicle stimulating hormone; FT3 = free triiodothyronine; FT4 = free thyroxine; (G) = gavage; Gastro = gastrointestinal; GD = gestational day; GH = growth hormone; GN = gross necropsy; GnRH = gonadotropin-releasing hormone; (GO) = gavage in oil; HDL = high density lipoprotein; HE = hematology; Hemato = hematological; HP = histopathology; IFN-γ = interferon gamma; IGF-1 = insulin-like growth factor-1; IL = interleukin; Immuno = immunological; (IN) = ingestion; IX = immunotoxicity; LABC = levator ani/bulbocavernosus; LDL = low density lipoprotein; LE = lethality; LH = luteinizing hormone; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; LSE = levels of significant exposure; M = male(s); MCP-1 = monocyte chemoattractant protein-1; Musc/skel = musculoskeletal; Neuro = neurological; NX = neurotoxicity; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OP = ophthalmology; OVA = ovalbumin; OW = organ weight; PCV = packed cell volume; PND = postnatal day; PNW = postnatal week; PPS = preputial separation; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; T3 = triiodothyronine; T4 = thyroxine; TNF-α = tumor necrosis factor-alpha; TRH = thyrotropin-releasing hormone; TSH = thyroid stimulating hormone; TT3 = total triiodothyronine; TT4 = total thyroxine; TWA = time-weighted average; UR = urinalysis; (W) = drinking water; WI = water intake

Figure 2-3. Levels of Significant Exposure to DEHP – Oral
Acute (≤14 days)

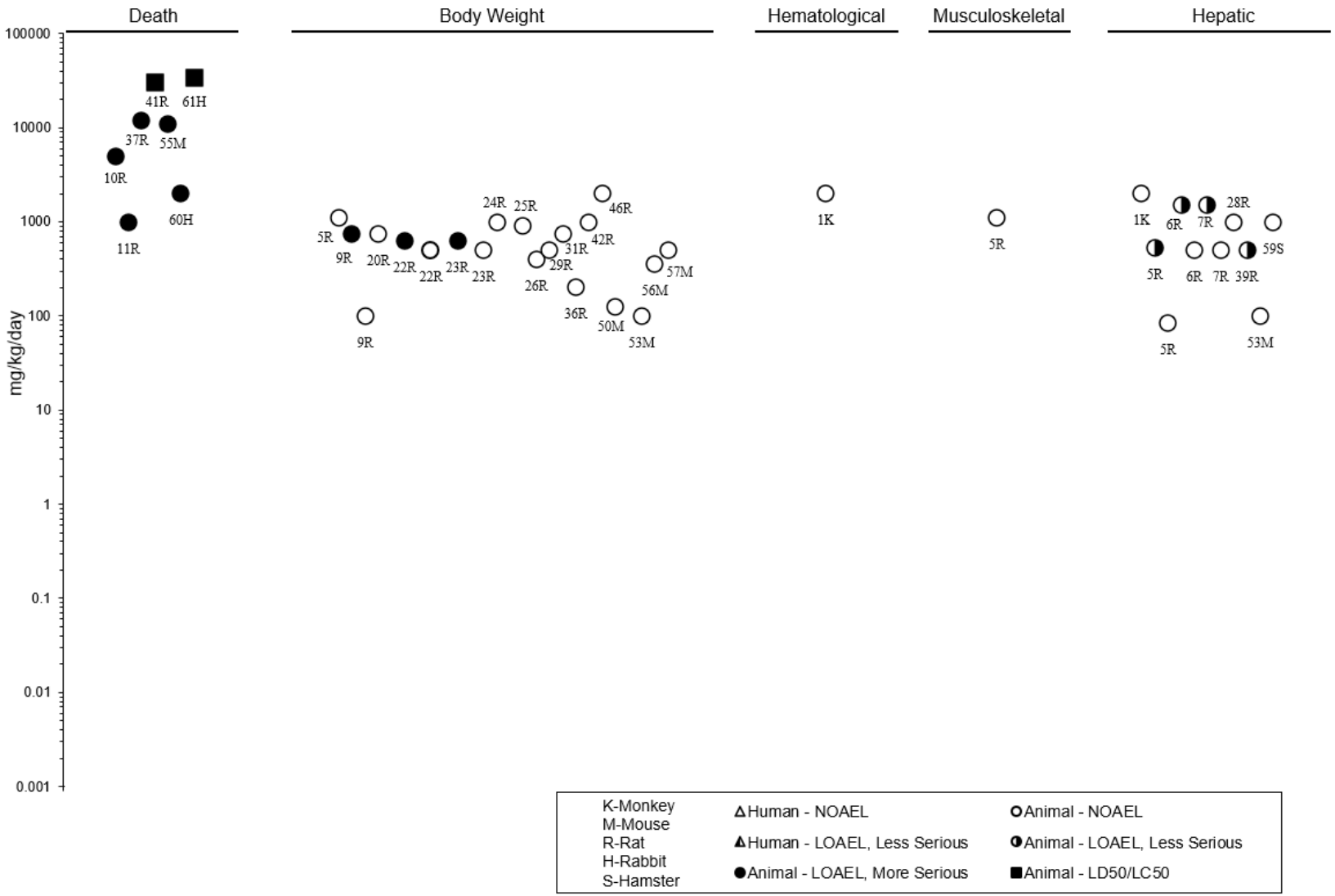


Figure 2-3. Levels of Significant Exposure to DEHP – Oral
Acute (≤14 days)

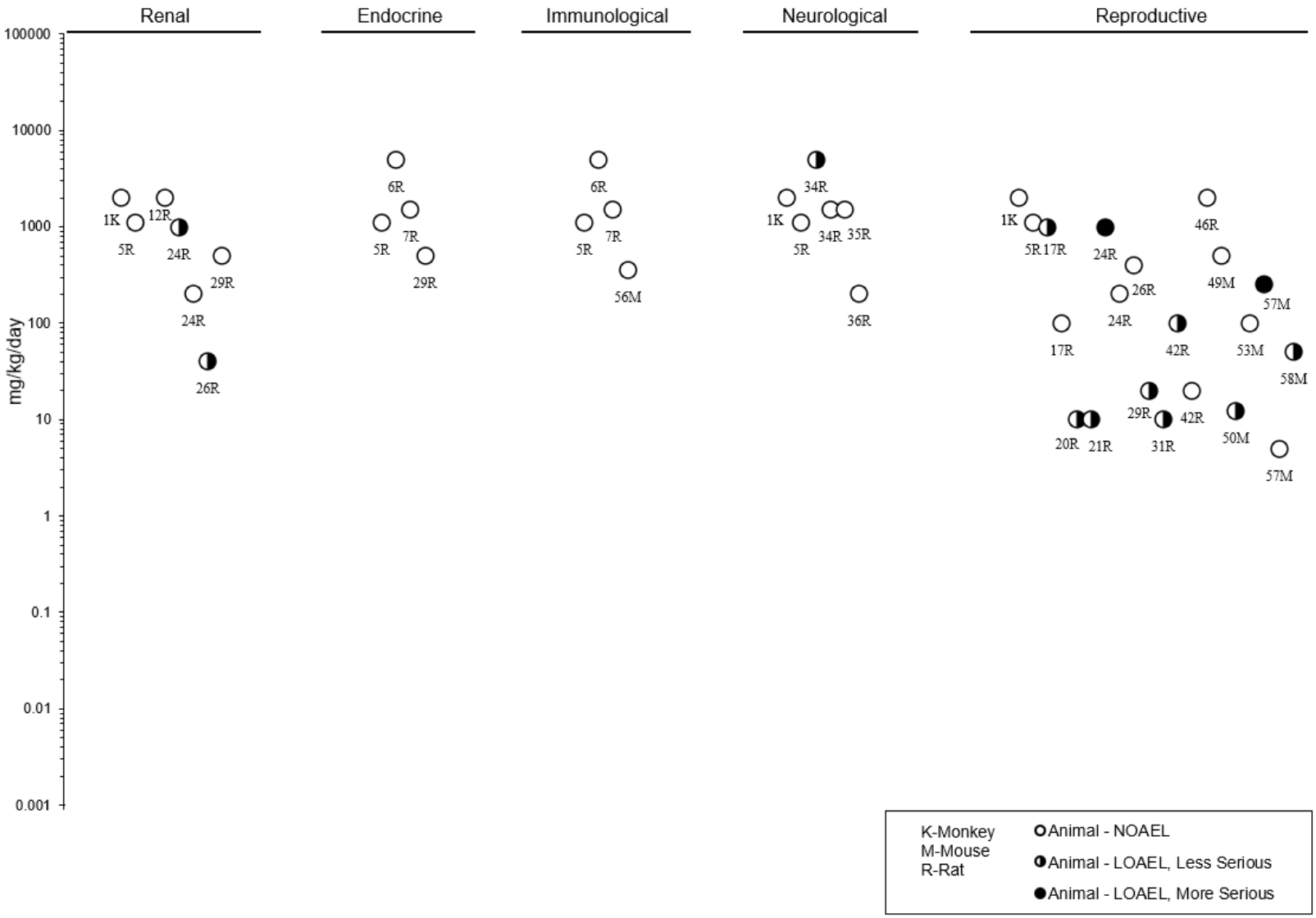


Figure 2-3. Levels of Significant Exposure to DEHP – Oral
Acute (≤14 days)

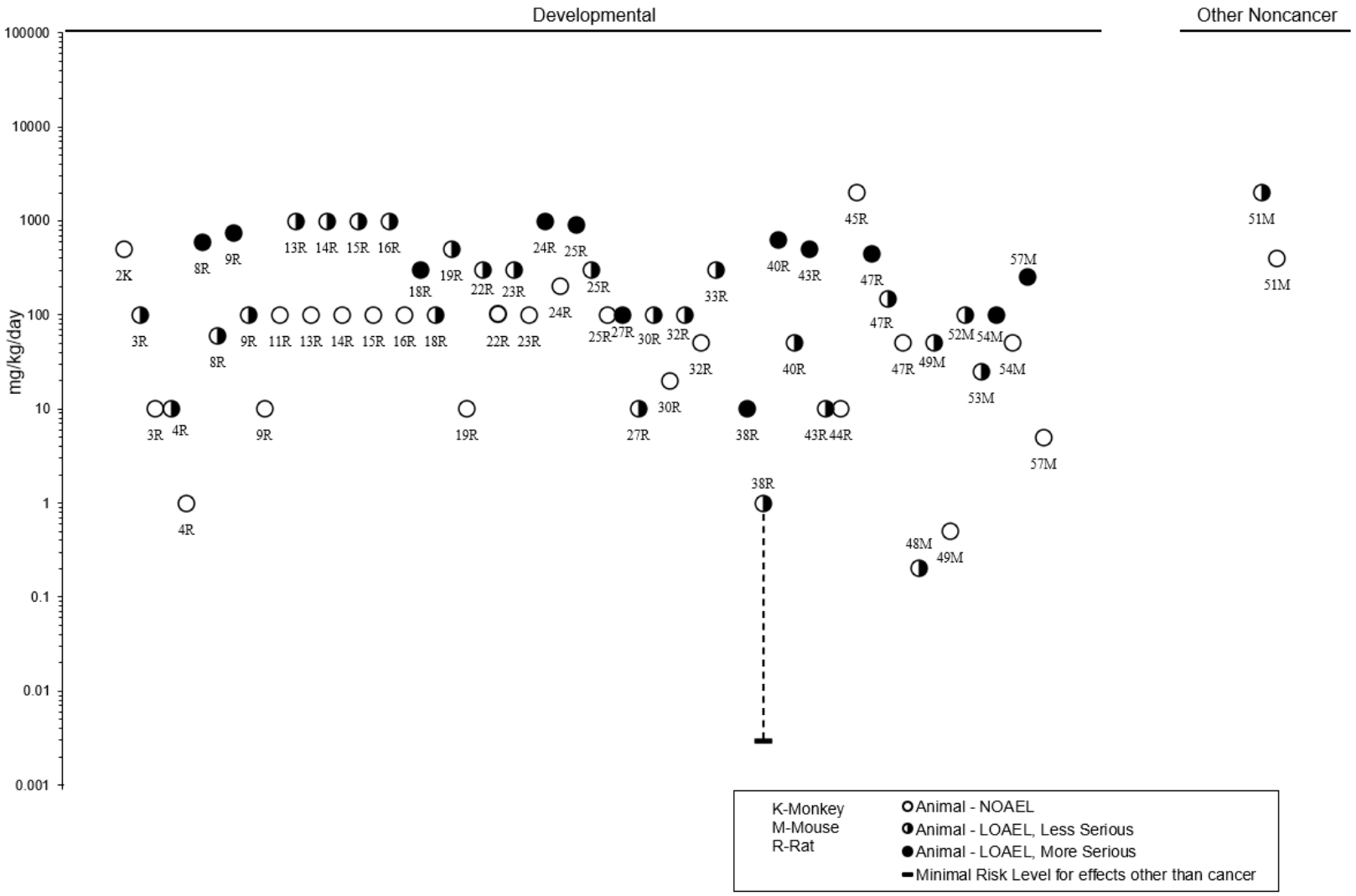


Figure 2-3. Levels of Significant Exposure to DEHP – Oral
Intermediate (15-364 days)

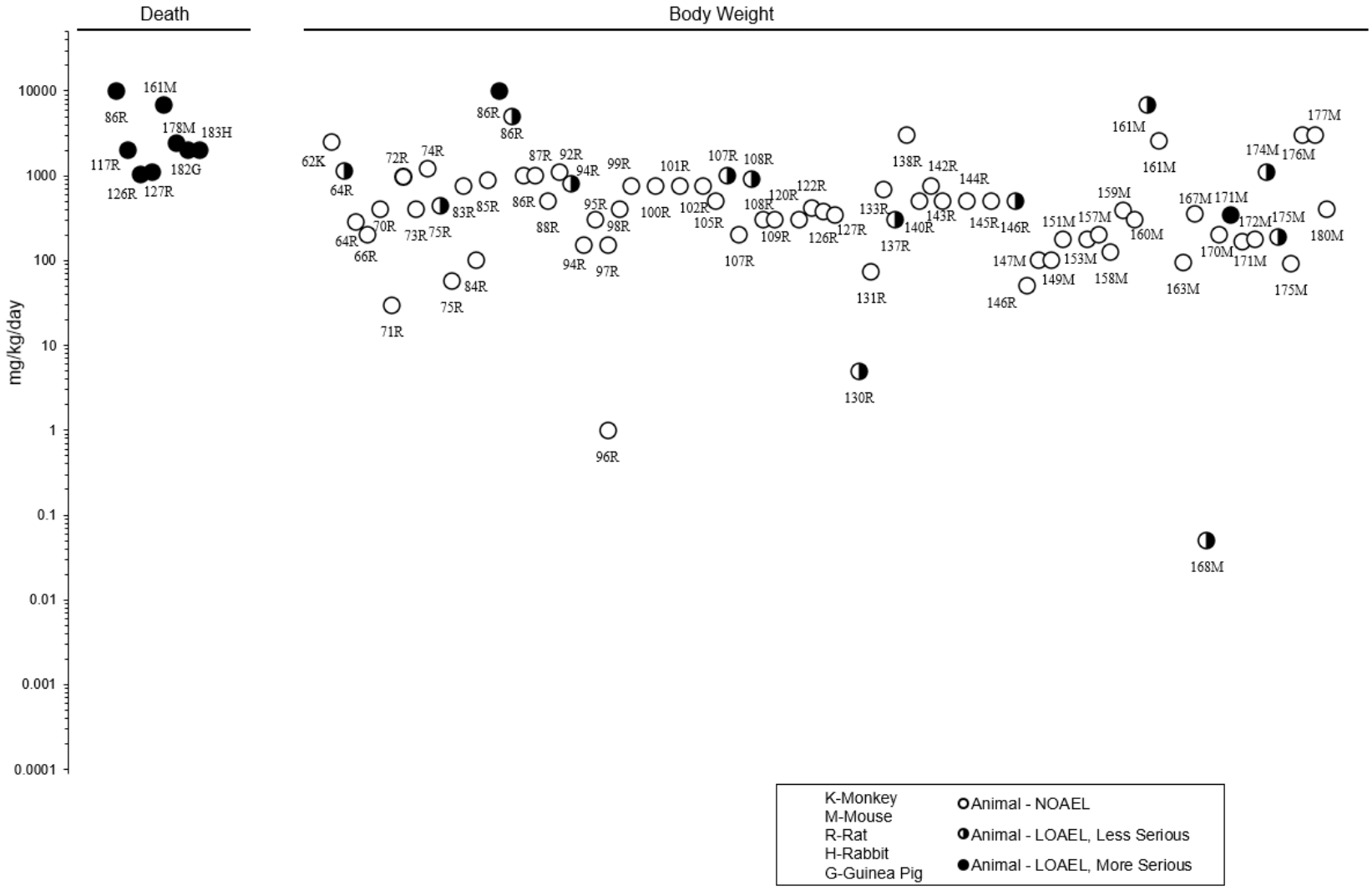


Figure 2-3. Levels of Significant Exposure to DEHP – Oral
Intermediate (15-364 days)

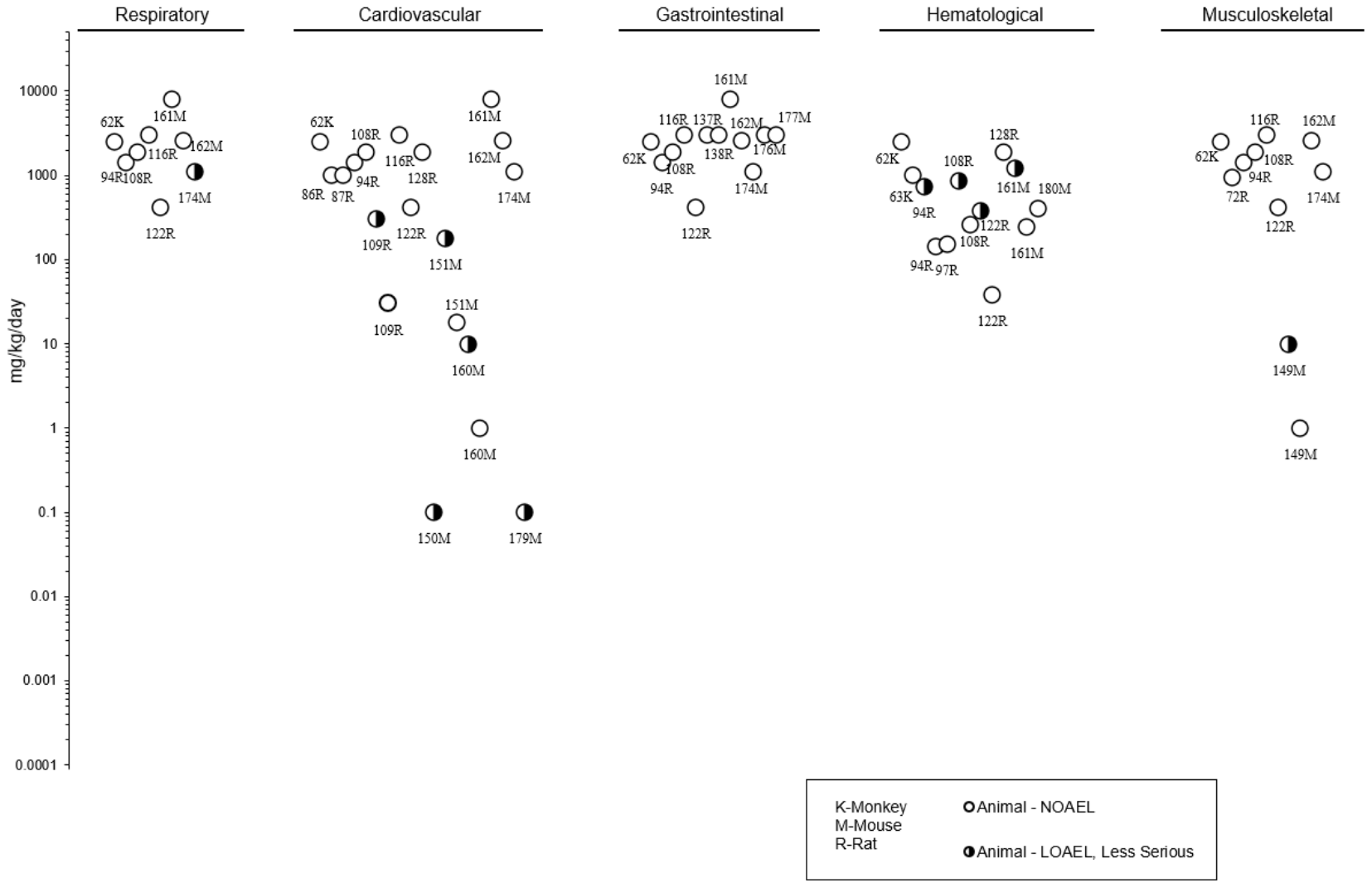


Figure 2-3. Levels of Significant Exposure to DEHP – Oral
Intermediate (15-364 days)

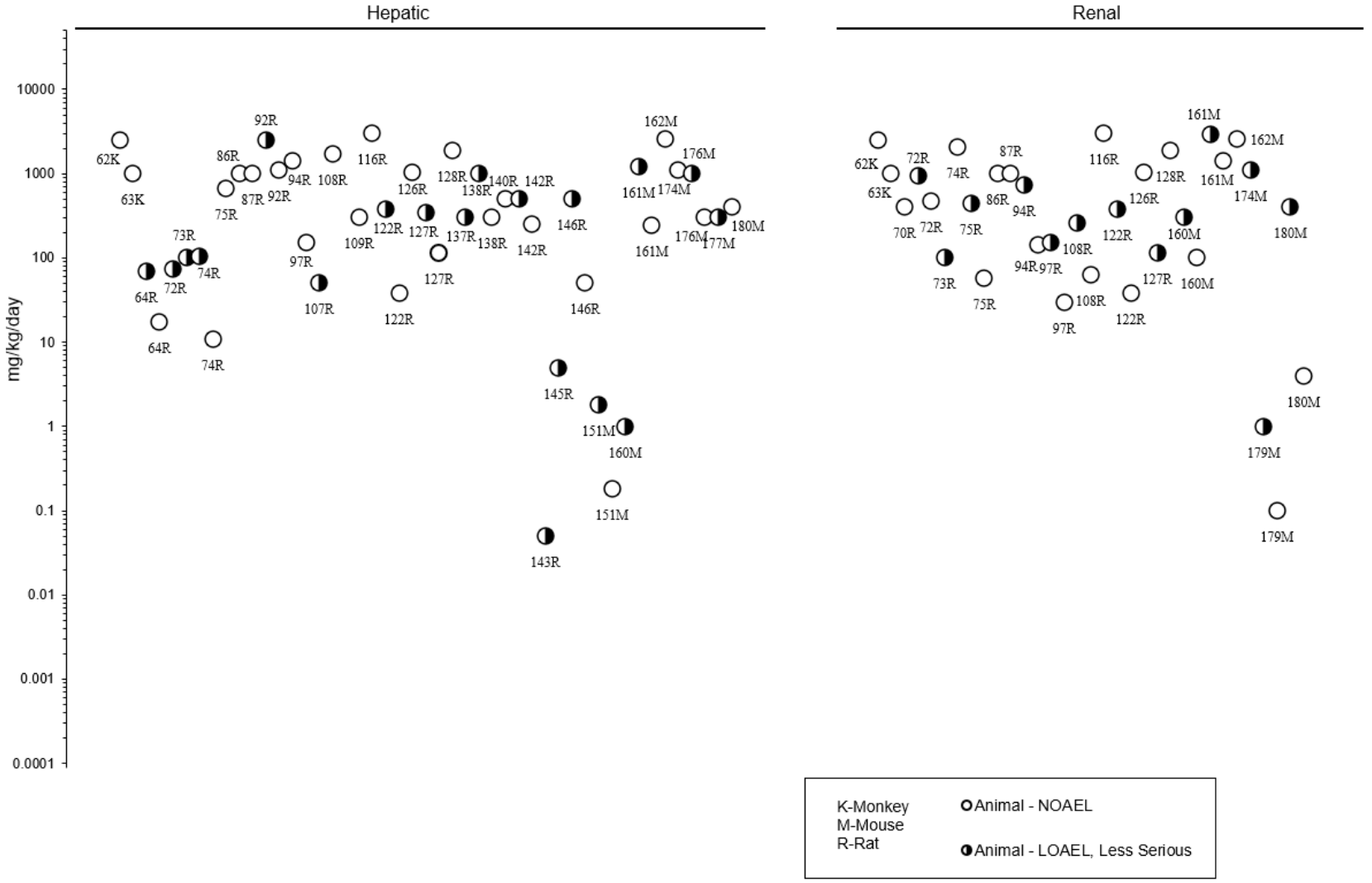


Figure 2-3. Levels of Significant Exposure to DEHP – Oral
Intermediate (15-364 days)

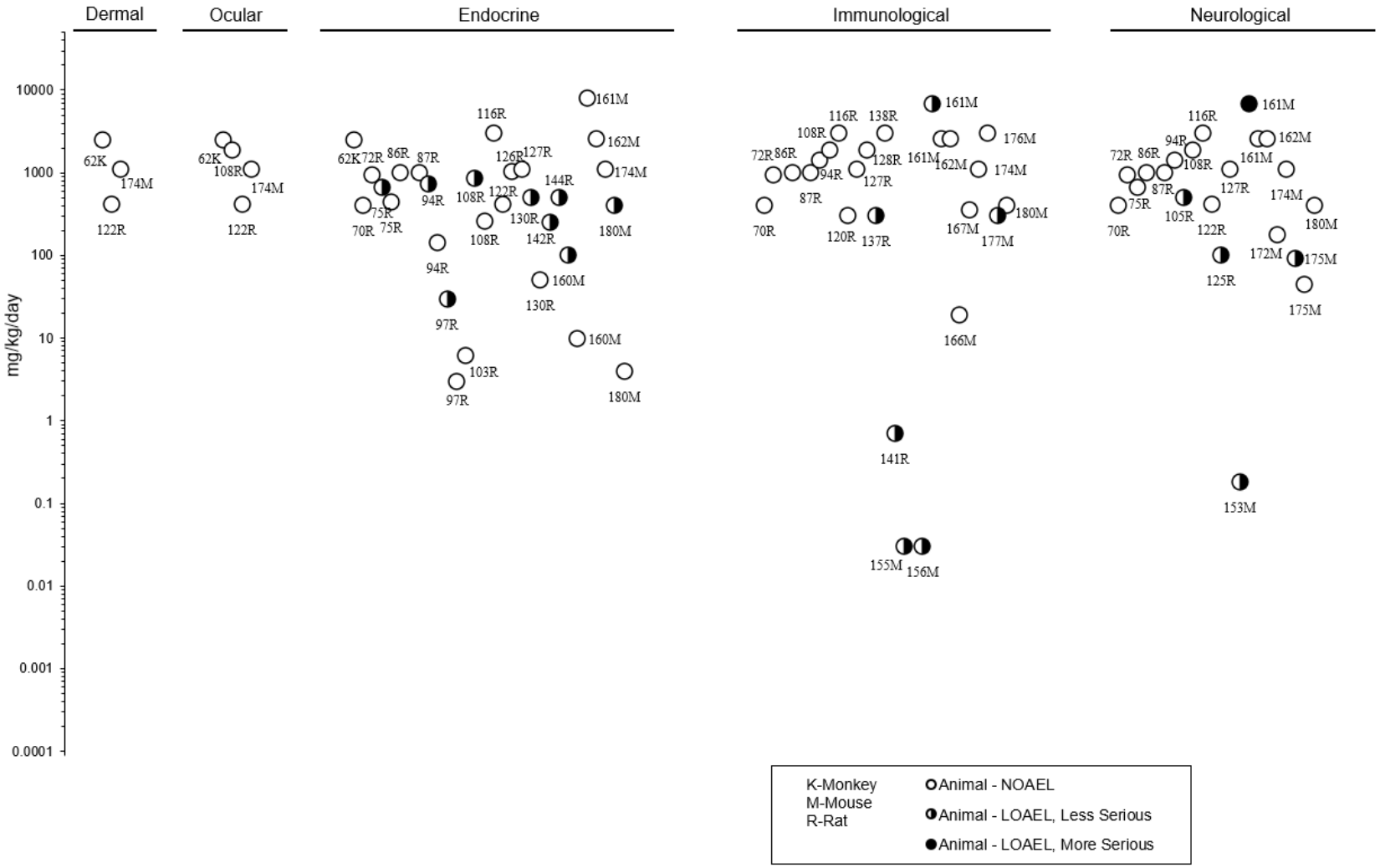


Figure 2-3. Levels of Significant Exposure to DEHP – Oral
Intermediate (15-364 days)

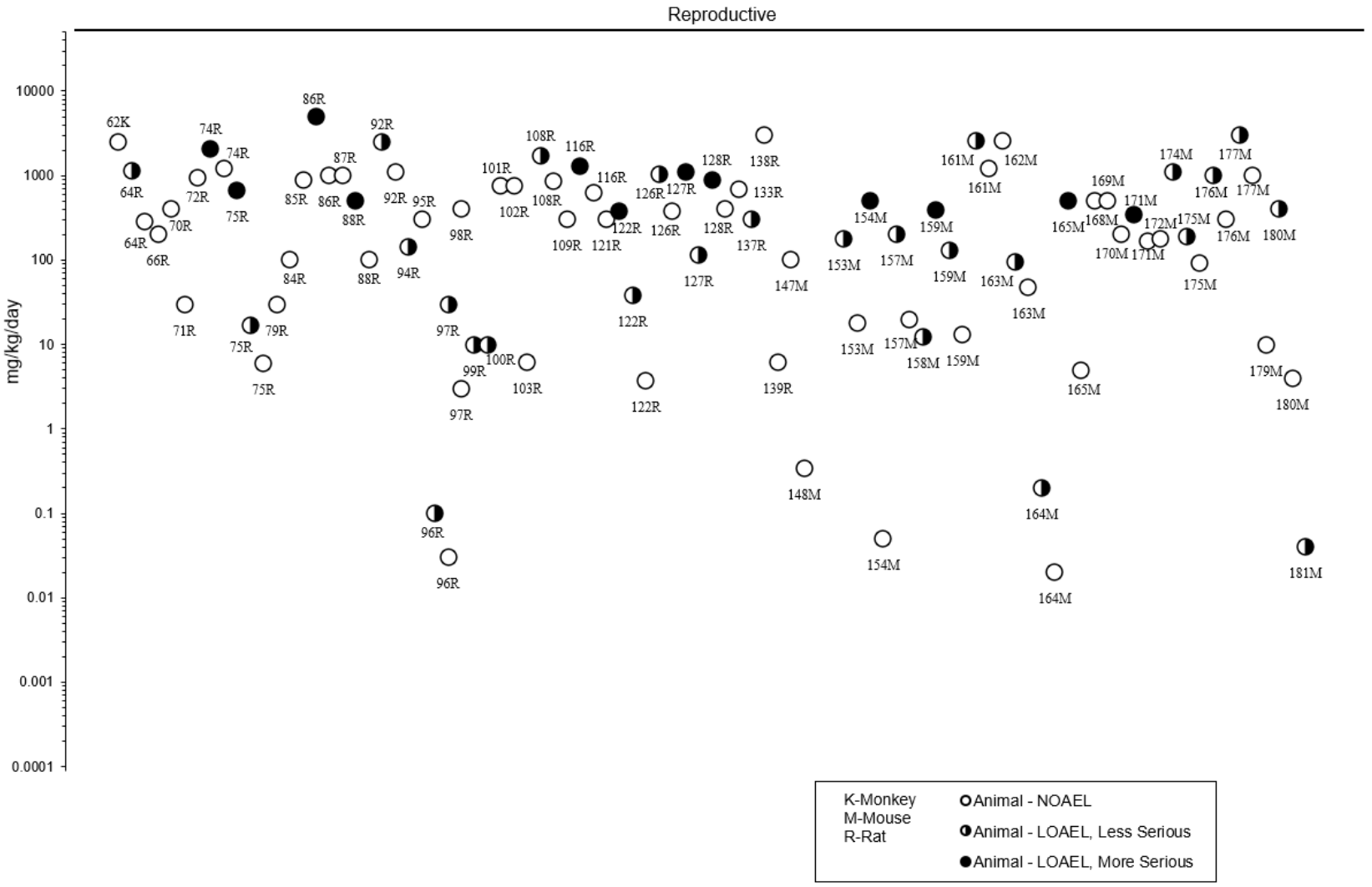


Figure 2-3. Levels of Significant Exposure to DEHP – Oral
Intermediate (15-364 days)

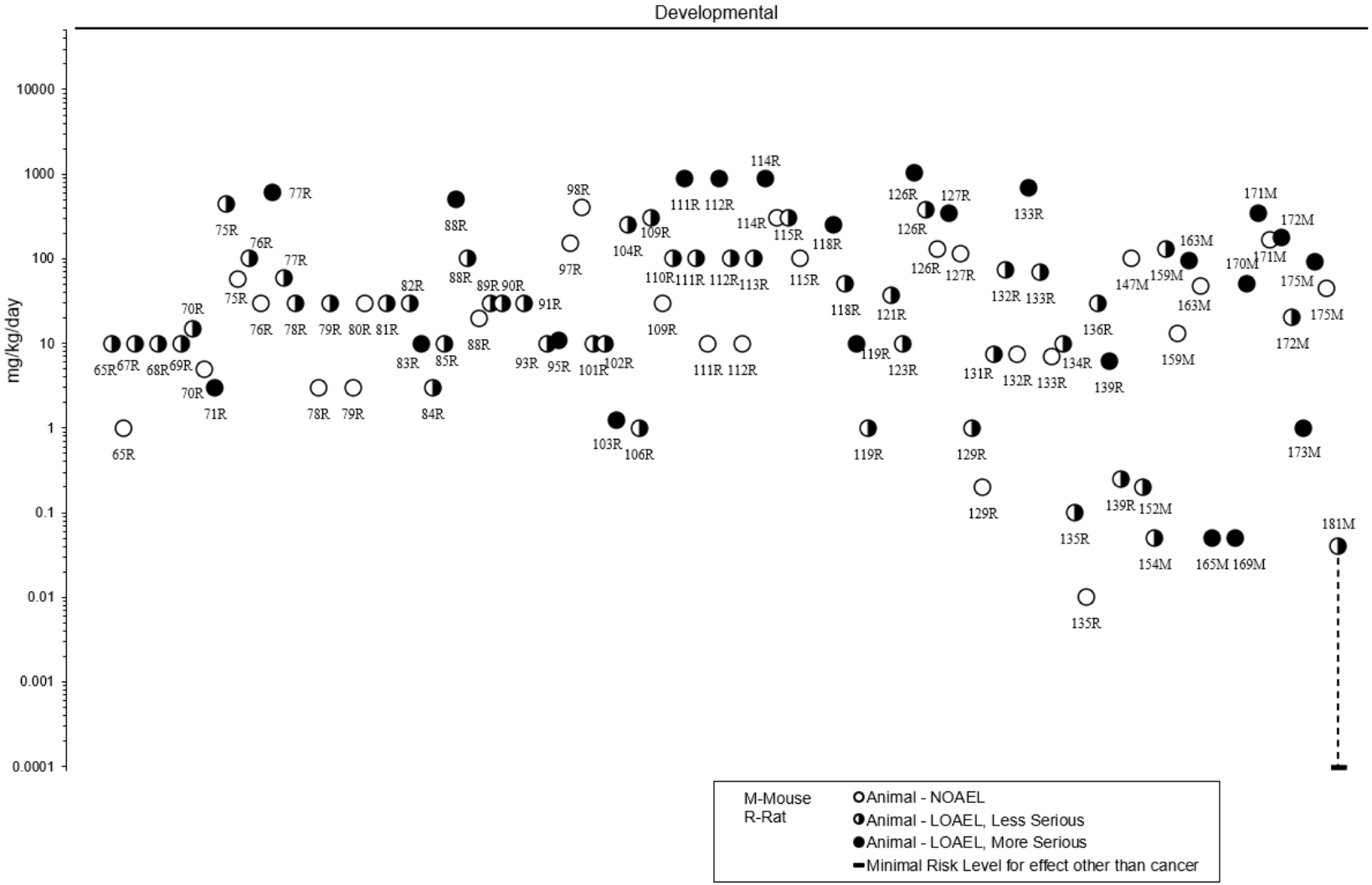


Figure 2-3. Levels of Significant Exposure to DEHP – Oral
Intermediate (15-364 days)

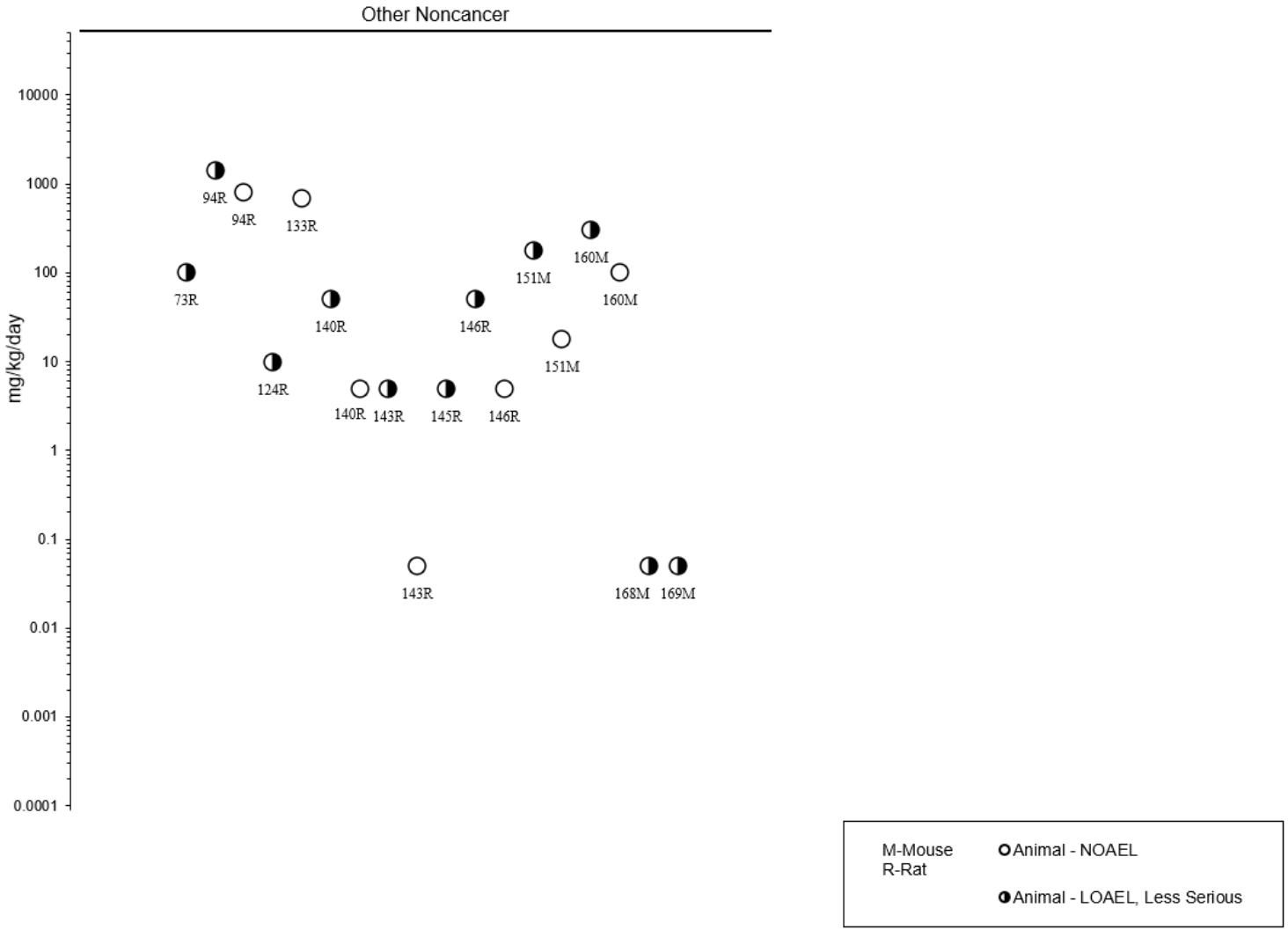


Figure 2-3. Levels of Significant Exposure to DEHP – Oral

Chronic (≥365 days)

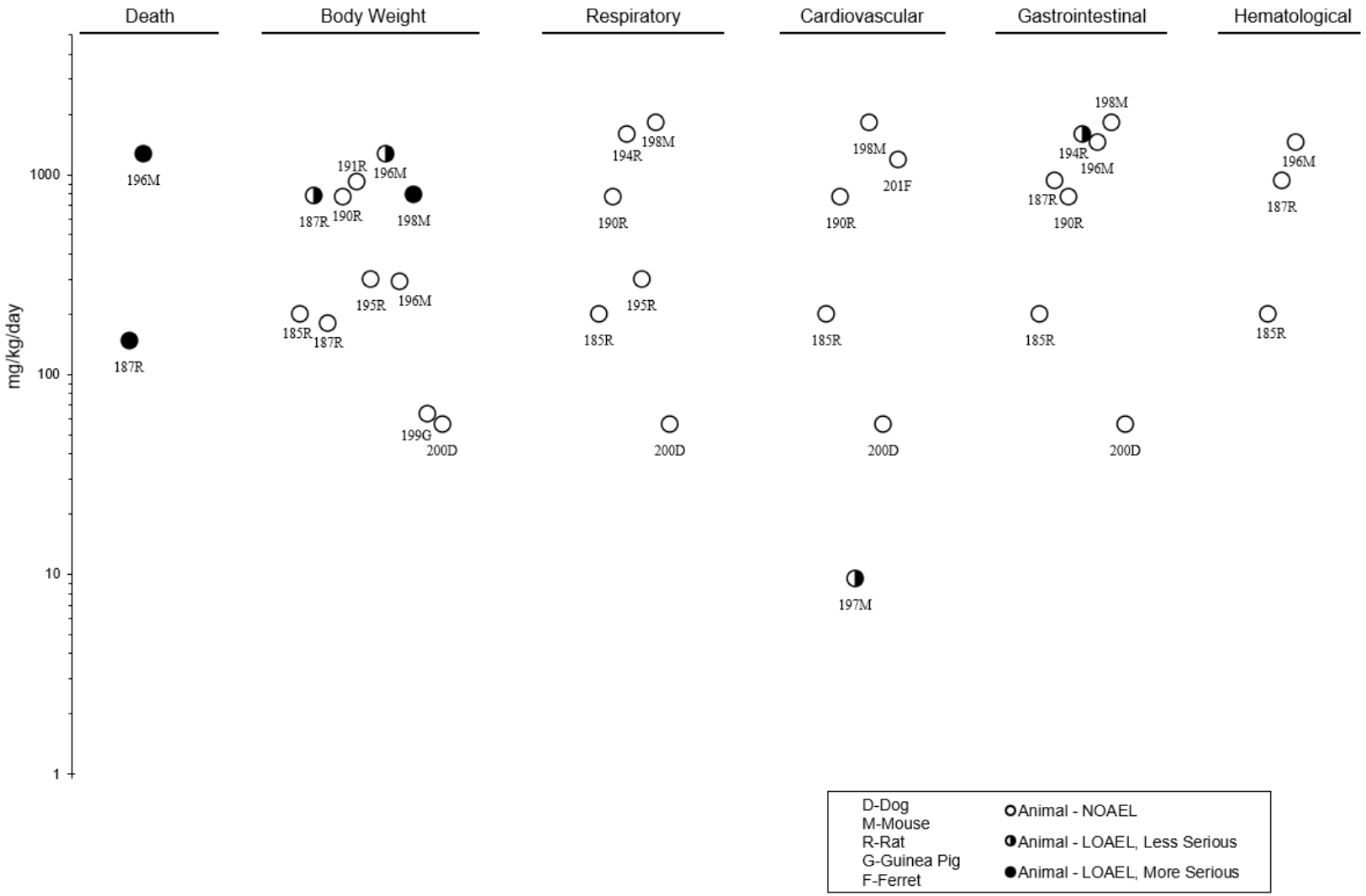


Figure 2-3. Levels of Significant Exposure to DEHP – Oral
Chronic (≥365 days)

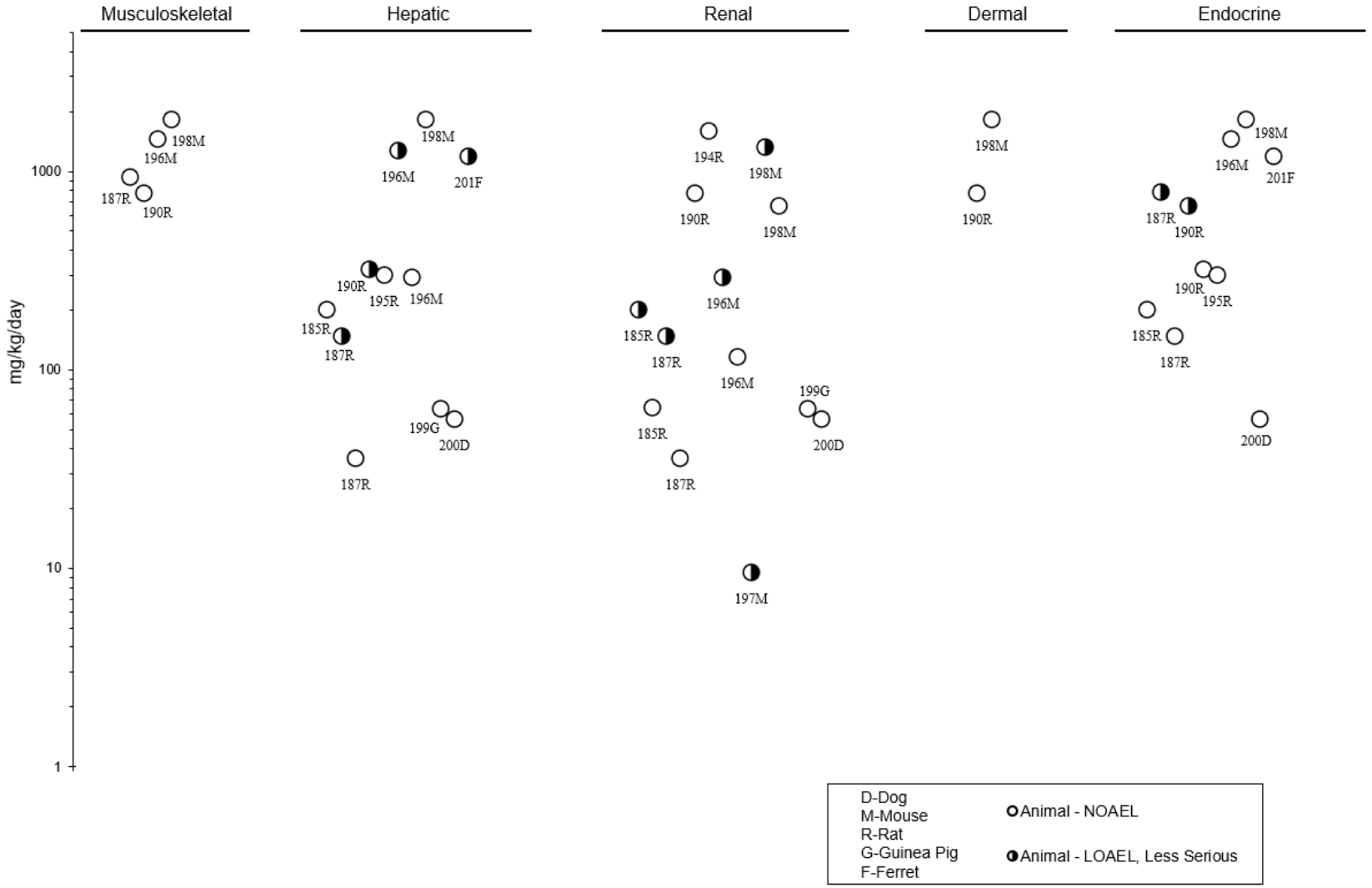
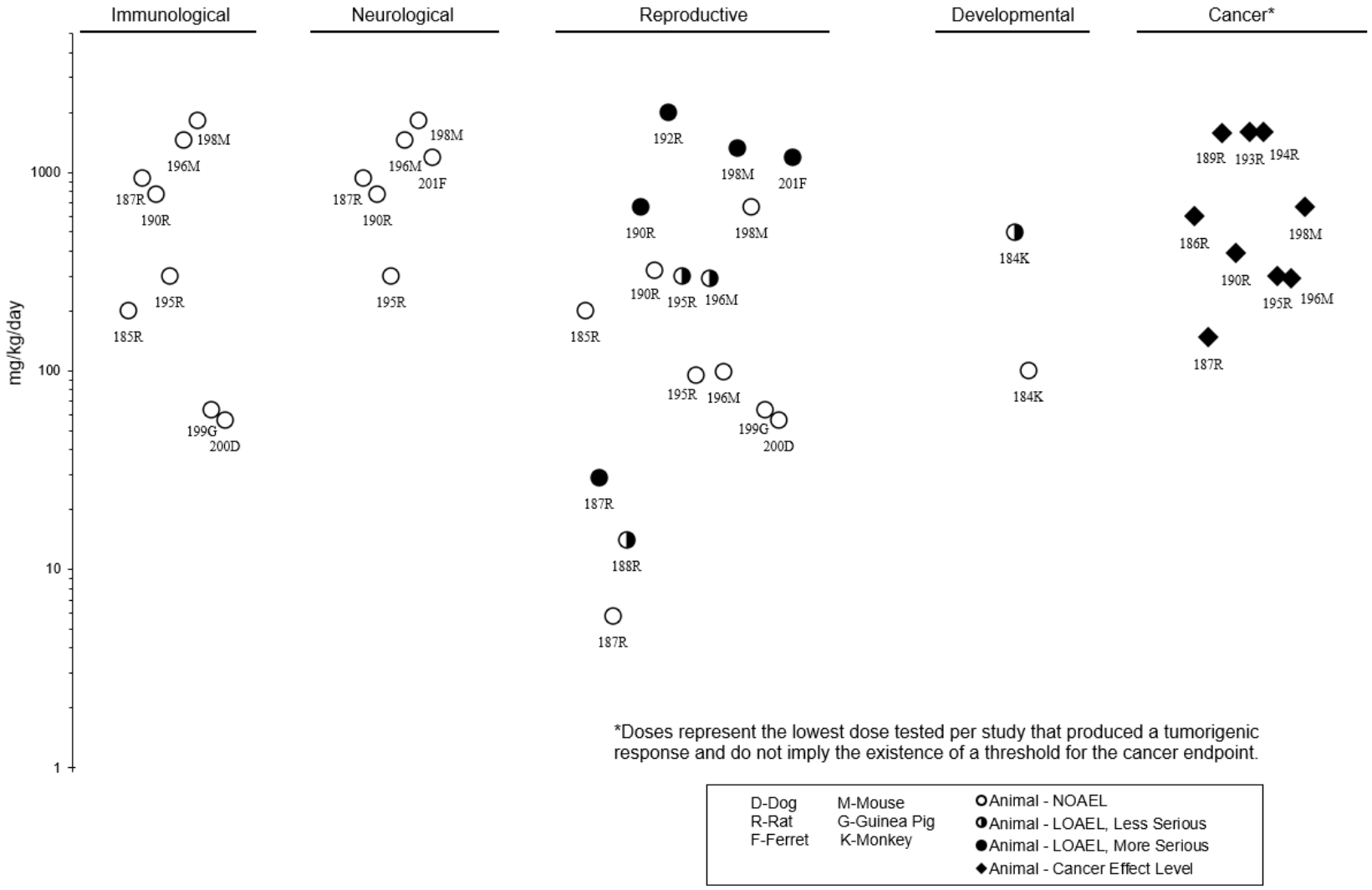


Figure 2-3. Levels of Significant Exposure to DEHP – Oral

Chronic (≥365 days)



2.2 DEATH

No studies were located regarding lethality in humans after inhalation exposure to DEHP. Studies in animals suggest that DEHP has low toxicity when inhaled. No deaths occurred in rats exposed to concentrations up to 21 ppm for 6 hours/day for 10 days (Merkle et al. 1988) or hamsters exposed to 0.0001 ppm for their lifetime (Schmezer et al. 1988). At a concentration of 0.0001 ppm, DEHP is present as a vapor, while at 21 ppm, it is an ultra-fine aerosol.

A single oral exposure to doses up to 10 g DEHP was not lethal to humans (Shaffer et al. 1945), and no case of death in humans after oral exposure to DEHP was identified in the available literature, suggesting that DEHP may not be acutely lethal to humans. This is supported by studies in rats and rabbits that indicate that single dose oral LD_{50} values are quite high (30,600–33,900 mg/kg) (Shaffer et al. 1945). To receive an equivalent dose, an adult human weighing 70 kg would have to consume about 4–5 pounds of DEHP. Some species seem to be more sensitive than others, potentially due to differences in toxicokinetics, as discussed in Section 3.1.6 (Animal-to-Human Extrapolations). In adult animals, exposure to 2,000 mg/kg/day (only dose tested) for up to 7 days resulted in mortalities in rabbits, but not in guinea pigs, mice, or rats (Parmar et al. 1988). After 2–4 weeks of exposure, deaths were observed at doses ≥2,000 mg/kg/day in rabbits, rats, and guinea pigs and 6,922 mg/kg/day in mice (Dalgaard et al. 2000; Myers 1992a; Parmar et al. 1987, 1988). Treatment of lactating female rats (postpartum days 1–7) with 5,000 mg DEHP/kg by gavage resulted in 25% mortality within 1 week of treatment (Cimini et al. 1994).

Deaths occurred at lower doses in longer-duration animal studies. In 2-generation studies, increased mortality was observed in F1 rats at doses of approximately 1,040–1,088 mg/kg/day; however, mortality rate was not increased above controls at doses ≤380 mg/kg/day (Schilling et al. 1999, 2001). In a 24-week dietary study, 100% mortality was observed after 16 weeks in mice exposed to doses of approximately 2,400 mg/kg/day in the diet (Ward et al. 1988); at the time of death, mean body weights were approximately 50% that of controls. In 2-year studies, survival was reduced in male F344 rats (12% less than controls) and male B6C3F1 mice (45% less than controls) that ingested 147 and 1,266 mg DEHP/kg/day in the diet, respectively (David et al. 1999, 2000a, 2000b). The most frequent cause of death in the chronic studies was mononuclear cell leukemia in the rats and liver tumors in the mice.

Certain populations, such as the young, may have increased susceptibility to DEHP-related mortality; however, the reason(s) why are not clear. Five doses of 2,000 mg DEHP/kg caused a 96% mortality in

rats ≤21 days old, but there were no deaths in rats ≥42 days old (Dostal et al. 1987). Increased mortality (60%) was also observed in sexually immature rats and mice exposed to dietary doses of ≥11,000 mg/kg/day for 14 days (NTP 1982).

When rabbits were exposed to single dermal applications at doses up to 20 mL/kg (19,700 mg/kg) DEHP using a modification of the U.S. Food and Drug Administration (FDA) cuff test, two of six rabbits in the highest dose group died. The dermal LD_{50} value calculated from these data was 25 mL/kg (24,600 mg/kg) (Shaffer et al. 1945).

2.3 BODY WEIGHT

Overview. Many epidemiological studies, primarily cross-sectional in design, have examined associations between DEHP exposure (measured as urinary metabolites) and anthropometric measurements relating to body weight, such as BMI, waist circumference, and risk of obesity or being overweight. A systematic review of phthalate exposure (including DEHP) and obesity outcomes conducted by Goodman et al. (2014) evaluated studies published through June, 2013. Numerous inhalation and oral animal studies have evaluated body weight following exposure to DEHP for various durations. Potential mechanisms of obesity have been evaluated in a review by Kim and Park (2014). Studies evaluating weight after developmental exposure (e.g., birth weight) are discussed in Section 2.17 (Developmental).

Epidemiology Studies. The systematic review conducted by Goodman et al. (2014) concluded that the available data (through June, 2013) evaluating obesity outcomes and phthalate exposure did not indicate a consistent association between DEHP and BMI, waist circumference, or fat distribution.

Studies published after Goodman et al. (2014) that met inclusion criteria (Appendix B) are shown in Table 2-3; these include a cohort study (Teitelbaum et al. 2012) where exposure was measured approximately 1 year prior to anthropometric measurements; 2 cohort studies where exposure was measured in pregnant women during the first trimester and body weights were measured at first and second trimester visits (Bellavia et al. 2017) or at delivery and 1 year postpartum (Perng et al. 2020); and 16 cross-sectional or case-control studies that measured exposure and outcome at the same time. Eleven additional cohort studies evaluating potential associations between growth or obesity in children and prenatal exposure (maternal urinary metabolites) are discussed in Section 2.17 (Developmental), as this study design evaluates potential effects of exposure during early development.

Table 2-3. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Bellavia et al. 2017 Cohort/cross-sectional, 347 pregnant women with full-term births, United States (Boston)	Gestational BWG or BMI	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	IQR: 0.2–0.8 µmol/L (SG-adj)	↔
Buser et al. 2014 Cross-sectional, children and adolescents (age 6–19 years) and nonpregnant, nonlactating adults (age >19 years), subject number not reported, United States	**Obesity (BMI ≥30) in adults**	**ΣDEHP**	GM (SE): 0.18 (0.01) µmol/mL	↑
		MEHP	GM (SE): 2.01 (0.10) ng/mL	↔
		MEHHP	15.86 (0.85)	↑
		MEOHP	9.16 (0.47)	↑
		MECPP	24.30 (1.20)	↑
	Overweight (BMI 25–29.9) in adults	ΣDEHP, MEHP, MEHHP, MEOHP, MECPP	See above	↔
	Obesity or overweight in children and adolescents	ΣDEHP	GM (SE): 0.24 (0.01) µmol/mL	↔
		MEHP	GM (SE): 2.18 (0.11) ng/mL	↔
		MEHHP	21.03 (1.25)	↔
		MEOHP	12.92 (0.72)	↔
		MECPP	34.79 (1.66)	↔
Dirtu et al. 2013 Case-control, 152 obese and 43 non-obese individuals, Belgium	**WC in controls**	ΣDEHP	IQR: 27–53 ng/mL	↔
		MEHP	2–5	↔
		MEHHP	9–19	↔
		MEOHP	3–9	↓
		MECPP	12–20	↓

Table 2-3. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
	WC in cases	ΣDEHP	30–61	↔
		MEHP	2–5	↔
		MEHHP	10–25	↔
		MEOHP	4–11	↔
		MECPP	12–22	↔
Hatch et al. 2008 Cross-sectional, 2,118 females and 2,251 males (age 6–80 years), United States (NHANES)	**BMI (females)**	**MEHP**	Ages 6–11: GM (SD): 5.4 (2.8) µg/g Cr	↔
			Ages 12–19: 3.8 (2.9)	↔
			Ages 20–59: 4.0 (2.9)	↔
			Ages 60–80: 3.3 (2.9)	↓
		MEHHP	Ages 6–11: 39.6 (2.5)	↔
			Ages 12–19: 21.1 (2.6)	↔
			Ages 20–59: 18.3 (2.8)	↔
			Ages 60–80: 18.4 (2.7)	↔
		MEOHP	Ages 6–11: 27.5 (2.4)	↔
			Ages 12–19: 15.0 (2.4)	↔
			Ages 20–59: 12.5 (2.7)	↔
			Ages 60–80:12.4 (2.6)	↔
	WC (females)	**MEHP**	Ages 6–11: see above	↔
			Ages 12–19: see above	↓
			Ages 20–59: see above	↔
			Ages 60–80: see above	↓
		MEHHP, MEOHP	All ages (see above)	↔
	BMI or WC (males)	MEHP	Ages 6–11: 5.5 (3.1)	↔
			Ages 12–19: 2.7 (3.0)	↔
			Ages 20–59: 3.3 (3.2)	↔
			Ages 60–80: 2.5 (2.9)	↔

Table 2-3. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
		MEHHP	Ages 6–11: 39.1 (2.4)	↔
			Ages 12–19: 18.2 (2.8)	↔
			Ages 20–59: 16.6 (3.0)	↔
			Ages 60–80: 13.2 (2.9)	↔
		MEOHP	Ages 6–11: 26.6 (2.4)	↔
			Ages 12–19: 12.2 (2.8)	↔
			Ages 20–59: 10.6 (2.8)	↔
			Ages 60–80: 9.2 (2.7)	↔
Hou et al. 2015a, 2015b Cross-sectional, 270 children and adolescents (age 6.5–15 years) and 38 complainants involved in lawsuit regarding plasticizer-tainted foods (age 6.5–8 years), Taiwan	**BMI**	ΣDEHP	IQR: 100.74–237.19 ng/mL	↔
		MEHP	10.04–87.08	↔
		MEHHP	23.49–60.30	↑
		MEOHP	16.43–41.00	↔
		MECPP	31.70–77.63	↔
	Waist-to-hip (circumference) ratio	ΣDEHP, MEHP	See above	↔
		MEHHP, MEOHP, MECPP	See above	↑
	WC	ΣDEHP, MEHP, MEOHP, MECPP	See above	↔
		MEHHP	23.49–60.30	↑
James-Todd et al. 2016b Cross-sectional, 350 pregnant women with full-term births, United States (Boston)	BMI	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	Median Q4: 2.09 µmol/L (SG-adj)	↔

Table 2-3. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
James-Todd et al. 2016a Case-control, 965 cases with metabolic syndrome and 1,754 subjects without metabolic syndrome (age 20–80 years), United States (NHANES)	**Central obesity** (WC ≥102 cm in men or ≥88 cm in women)	**ΣDEHP** (MEHP, MEHHP, MEOHP)	Cases: GM (95% CI): 0.13 (0.12, 0.15) ng/mL Controls: 0.12 (0.10, 0.13)	↑
Kang et al. 2019 Cross-sectional, 4,752 adults (2,197 men, 2,555 women; age ≥19 years), Korea	**Obesity (BMI >30 kg/m^2) in women**	**ΣDEHP**	All subjects: GM (SE): 80.92 (1.42) µg/g Cr Normal/underweight: 76.81 (1.63) Overweight: 92.88 (2.64) Obese: 87.48 (6.65)	**All:** ↑ <50 yr: ↔ **≥50 yr:** ↑
		MEHHP	All subjects: 27.97 (0.54) Normal/underweight: 26.34 (0.61) Overweight: 32.69 (0.97) Obese: 31.02 (2.45)	**All:** ↑ <50 yr: ↔ **≥50 yr:** ↑
		MEOHP	All subjects: 20.08 (0.38) Normal/underweight: 19.17 (0.43) Overweight: 22.68 (0.68) Obese: 21.47 (1.75)	All: ↔ <50 yr: ↔ **≥50 yr:** ↑
		MECPP	All subjects: 31.69 (0.59) Normal/underweight: 30.19 (0.68) Overweight: 35.99 (1.09) Obese: 34.00 (2.53)	All: ↔ <50 yr: ↔ ≥50 yr: ↔
	Obesity (BMI >30 kg/m^2) in men	ΣDEHP	All subjects: 57.96 (1.06) Normal/underweight: 59.65 (1.32) Overweight: 55.78 (1.68) Obese: 55.80 (4.06)	All: ↔ <50 yr: ↔ ≥50 yr: ↔
		MEHHP	All subjects: 20.66 (0.42) Normal/underweight: 20.99 (0.52) Overweight: 20.12 (0.64) Obese: 20.85 (1.58)	All: ↔ <50 yr: ↔ ≥50 yr: ↔

Table 2-3. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
		MEOHP	All subjects: 13.76 (0.28) Normal/underweight: 14.27 (0.35) Overweight: 13.13 (0.44) Obese: 13.00 (1.04)	All: ↔ <50 yr: ↔ ≥50 yr: ↔
		MECPP	All subjects: 22.74 (0.40) Normal/underweight: 23.53 (0.50) Overweight: 21.83 (0.65) Obese: 21.24 (1.50)	All: ↔ <50 yr: ↔ ≥50 yr: ↔
Kim et al. 2018a Cross-sectional with nested case-control, 65 overweight girls (33 prepubertal, 32 pubertal) and 72 age-matched, non-overweight controls (35 prepubertal, 37 pubertal girls) (age 6–13 years), Korea	**Central obesity, BMI, WC, or percent body fat (prepubertal girls)**	MEHP	Overweight: GM (SE): 14.0 (2.9) µg/g Cr Control: 15.2 (2.5)	↔
		MEHHP	Overweight: 38.3 (15.6) Control: 41.5 (5.6)	↑
		MEOHP	Overweight: 29.7 (8.1) Control: 35.0 (4.5)	↔
		MECPP	Overweight: 82.8 (29.3) Control: 104.1 (1.7)	↔
	Central obesity, BMI, WC, or body fat (pubertal girls)	MEHP	Overweight: 13.2 (1.5) Control: 11.9 (1.4)	↔
		MEHHP	Overweight: 37.7 (5.8) Control: 37.7 (4.3)	↔
		MEOHP	Overweight: 29.7 (3.4) Control: 30.3 (3.5)	↔
		MECPP	Overweight: 90.9 (15.6) Control: 90.3 (14.4)	↔
Ko et al. 2019 Cross-sectional, 435 adults (388 men, 47 women; mean age 32.16 years), Taiwan	Abdominal obesity (WC ≥90 cm for men, ≥80 cm for women)	ΣDEHP	NR	↔
		MEHP	All: 25th–95th percentile: 0.269–2.789 µg/g Cr Men: 0.263–2.800 Women: 0.299–2.551	NR

Table 2-3. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
		MEHHP	All: 0.908–6.045 Men: 0.910–6.013 Women: 0.841–9.648	NR
		MEOHP	All: 0.486–2.603 Men: 0.479–2.636 Women: 0.505–2.509	NR
Li et al. 2020 Cross-sectional, 942 elderly adults (432 males, 510 females; age ≥60 years), China	**Obesity (BMI >28 kg/m^2)**	**ΣDEHP**	All: IQR: 4.45–15.32 µg/g Cr **Men:** 4.20–14.71 Women: 4.70–15.80	All: ↔ **Men:** ↑ Women: ↔
		MEHP	**All:** 0.15–1.73 Men: 0.16–1.82 Women: 0.14–1.64	**All:** ↑ Men: ↔ Women: ↔
		MEHHP	All: 1.44–4.23 **Men**: 1.32–3.97 Women: 1.53–4.51	All: ↔ **Men:** ↑ Women: ↔
		MEOHP	**All:** 1.27–3.78 **Men:** 1.23–3.39 **Women:** 1.33–4.13	**All:** ↑ **Men:** ↑ **Women:** ↑
	Central obesity (WC ≥85 cm men, WC ≥80 cm women)	**ΣDEHP**	See above	All: ↔ **Men:** ↑ Women: ↔
		MEHP	See above	**All:** ↑ Men: ↔ Women: ↔
		MEHHP	See above	All: ↔ Men: ↔ Women: ↔
		MEOHP	See above	All: ↔ **Men:** ↑ Women: ↔

Table 2-3. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Lin et al. 2016 Cross-sectional, 793 students including 303 with and 486 without elevated blood pressure in childhood (mean age 21.28 years), Taiwan	**BMI**	**MEHP**	IQR: 1.7–38.99 µg/g Cr	↑
		MEHHP	15.86–43.16	↔
		MEOHP	10.18–26.56	↔
Lin et al. 2020 Cross-sectional, 792 adolescents and adults (age 12–30 years), Taiwan	**BMI**	**MEHP**	GM (95% CI): 6.1 (5.1, 7.3) µg/g Cr	↑
		MEHHP	27.9 (26.1, 30.0)	↔
		MEOHP	17.5 (16.4, 18.5)	↔
Perng et al. 2020 Cohort, 199 pregnant women (mean age 27.87 years), Mexico	**Maternal weight at delivery** (accounting for early pregnancy weight status and fetal growth)	**ΣDEHP** (MEHP, MEHHP, MEOHP, MECPP)	IQR: 39.95–98.21 ng/mL	↓
	Rate of body weight loss during first year postpartum	**ΣDEHP**	See above	↓
Song et al. 2014 Cohort, 977 non-diabetic nurses (age 25–55 years) United States (NHANES)	BMI or weight gain	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	IQR: 115–870 nmol/L	↔
Stahlhut et al. 2007 Cross-sectional, 1,451 adult males (not taking insulin, oral hypoglycemic agents, or sex hormone agonists/antagonists), United States (NHANES)	**WC**	MEHP	Mean (SE): 11 (1.3) µg/g Cr	↔
		MEHHP	65.8 (7.9)	↑
		MEOHP	38.7 (4.5)	↑

Table 2-3. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Teitelbaum et al. 2012 Cohort, 379 Hispanic and Black children (299 girls, 80 boys; age 6–8 years), United States (New York)	BMI or WC	ΣDEHP	Girls: median: 235.5 µg/g Cr Boys: median: 251.2 µg/g Cr	↔
		MEHP	Girls: 6.5; boys: 6.3	↔
		MEHHP	Girls: 72.0; boys: 75.7	↔
		MEOHP	Girls: 44.8; boys: 50.4	↔
		MECPP	Girls: 114.2; boys:114.6	↔
Wang et al. 2013 Cross-sectional, 259 students (age 8–15 years), including normal weight (n=124), overweight (n=53), and obese (n=82) subjects; China	**BMI or WC**	ΣDEHP	GM: 117.3 ng/mL	↔
		MEHP	21.3	↑
		MEHHP	16.1	↔
		MEOHP	22.9	↔
		MECPP	28.8	↔
Yaghjyan et al. 2015a, 2015b Cross-sectional, 6,005 women (age ≥18 years), United States (NHANES)	**BMI**	ΣDEHP	IQR: 19.59–58.66 µg/g Cr	↔
		MEHP	1.49–5.95	↑
		MEHHP	9.86–31.09	↔
		MEOHP	6.83–19.84	↔
		MECPP	17.16–49.78	↔
	WC	ΣDEHP, MEHP, MEHHP, MEOHP	See above	↔
		MECPP	See above	↑
Zhang et al. 2014 Cross-sectional, 246 girls (age 8–13 years), China	**Obesity**	**ΣDEHP**	8–10 years: range: 5.2–497.7 ng/mL 11–13 years: 1.3–864.4	↑
		MEHP	8–10 years: <LOD–92.2 11–13 years: <LOD–117.1	↔
		MEHHP	8–10 years: 3.2–290.0 11–13 years: 0.8–508.4	↑
		MEOHP	8–10 years: 1.2–115.5 11–13 years: <LOD–238.8	↑

Table 2-3. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
	Overweight	ΣDEHP, MEHP, MEHHP, MEOHP	All ages (see above)	↔

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; BMI = body mass index; BWG = body weight gain; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; IQR = interquartile range; LOD = limit of detection; MECPP = mono(2-ethyl-5-carboxypentyl)phthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NHANES = National Health and Nutrition Examination Survey; NR = not reported; Q4 = quartile four (of exposure); SD = standard deviation; SE = standard error; SG-adj = specific gravity adjusted; WC = waist circumference

Perng et al. (2020) observed an association between first trimester urinary ∑DEHP metabolite levels and decreased maternal weight at delivery (after accounting for early pregnancy weight status and fetal growth); however, rate of body weight loss over the first year postpartum was decreased with increased first trimester urinary ∑DEHP metabolite levels. In the other pregnancy cohort, Bellavia et al. (2017) observed an inverse U-shaped relationship between first trimester urinary ∑DEHP metabolite levels and early gestational weight gain (between first and second trimesters). In a cross-sectional analysis of the same cohort, urinary ∑DEHP metabolite levels were associated with higher first trimester BMI (Bellavia et al. 2017). In other cross-sectional and case-control studies, associations with DEHP urinary metabolite levels were reported for increased BMI in adults (Lin et al. 2016, 2020; Yaghjyan et al. 2015a, 2015b) and adolescents and children (Hou et al. 2015a, 2015b; Kim et al. 2018a; Lin et al. 2020; Wang et al. 2013), waist circumference in children (Hou et al. 2015a, 2015b; Kim et al. 2018a; Wang et al. 2013), and increased odds of obesity and/or central obesity in adults (Buser et al. 2014; James-Todd et al. 2016a; Kang et al. 2019; Li et al. 2020) or children (Kim et al. 2018a). Three studies reported lower obesity with higher DEHP metabolite levels. Yaghjyan et al. (2015a, 2015b) reported decreased odds of increased waist circumference in adult women; Zhang et al. (2014) observed lower odds of obesity (weight >90th percentile) in children aged 8–13 years; and Dirtu et al. (2013) reported negative associations between waist circumference and DEHP metabolite levels. A few studies did not observe an association between anthropometric measurements and DEHP exposure in adults (Ko et al. 2019), pregnant women (James-Todd et al. 2016b), or children (Teitelbaum et al. 2012).

The epidemiological data on DEHP metabolite levels and obesity parameters may be confounded by covariation among body weight, caloric intake, dietary composition (e.g., processed versus unprocessed foods), urinary creatinine levels, and DEHP exposure. As discussed in Section 5.6, diet is the primary source of exposure to DEHP. Individuals with higher body weight may experience higher caloric intake, leading to higher DEHP exposure. This relationship could lead to correlations between urinary metabolite levels and BMI or waist circumference that stem from higher caloric (and DEHP) intake rather than an effect of DEHP on these endpoints. By considering caloric intake as a covariate, confounding can be minimized; studies that considered caloric intake include Teitelbaum et al. (2012), James-Todd et al. (2016a), Yaghjyan et al. (2015a, 2015b), and Buser et al. (2014).

The use of urinary creatinine levels to correct for dilution of metabolite levels may also confound the data pertaining to BMI and waist circumference. Creatinine is a breakdown of muscle metabolism, and its levels in urine depend upon factors such as muscle mass, gender, age, and diet (among other factors; Johns et al. 2015). Because urinary creatinine levels are correlated to BMI and muscle mass

independently of phthalate exposure (Johns et al. 2015), studies that used creatinine-corrected metabolite levels to assess associations with BMI or similar metrics (Kang et al. 2019; Kim et al. 2018a; Li et al. 2020; Lin et al. 2016, 2020; Yaghjyan et al. 2015a, 2015b) or reported results after adjustment for urinary creatinine (Buser et al. 2014; Hou et al. 2015a, 2015b; James-Todd et al. 2016a; Teitelbaum et al. 2012) may yield spurious results for BMI or waist circumference. Studies that did not account for dilution by creatinine or specific gravity correction, or by consideration of one of these as a covariate in modeling (Dirtu et al. 2013; Zhang et al. 2014), may also be biased due to the lack of consideration of dilution. In their systematic review, Goodman et al. (2014) noted that positive associations between phthalates and obesity or overweight measures were most often seen in studies that did not account for urinary dilution of metabolite levels.

Animal Studies. In adult rats, no body weight effects were observed following nose-only exposure to concentrations up to 63 ppm for 4 weeks (5 days/week, 6 hours/day) (Klimisch et al. 1991, 1992). In mice, no body weight effects were observed in females intermittently exposed to concentrations up to 0.81 ppm for 14 weeks (20 minutes/day; 5 days/week for the first 2 weeks, 1 day/week for the next 12 weeks) (Larsen et al. 2007).

Numerous studies have documented reductions in body weight gain in rodents following oral exposure to high doses. However, dietary studies are complicated by evidence of decreased palatability at high doses, resulting in decreased food consumption. Due to this, gavage, drinking water, and dietary studies are discussed separately below. Body weight effects reported at dietary doses associated with decreased food consumption of a similar magnitude are not considered LOAELs in Table 2-2; however, since the relative contribution of decreased food intake cannot be fully determined, these values are also not listed as NOAELs. Body weight effects reported from dietary studies in the absence of food consumption data are also not reported as LOAELs in Table 2-2 since the potential impact of palatability cannot be assessed. However, all findings are discussed below.

Gavage studies in rodents. Numerous gavage studies in rodents did not report exposure-related changes in body weight in nonpregnant, adult rodents at acute doses ≤2,000 mg/kg/day or intermediate-duration doses ≤1,000 mg (Table 2-2). The only intermediate-duration study that tested gavage doses >1,000 mg/kg/day reported a 9–32% decrease in body weight in male Wistar rats exposed to 5,000–10,000 mg/kg/day for 4 weeks (Dalgaard et al. 2000). In contrast, two intermediate-duration studies reported body weight increases ≥10% in Wistar rats exposed to doses ≥5 mg/kg/day for 4 weeks (Sun et

al. 2018) or 500 mg/kg/day for 8 weeks (Zhou et al. 2019). No chronic-duration gavage studies in rodents were identified.

In pregnant animals, Sprague-Dawley or Long-Evans rats exposed to ≥625 mg/kg/day via gavage from gestation day (GD) 14 to 18, body weight gain decreases >30% were observed; actual body weight data were not reported (Hannas et al. 2011). Another Sprague-Dawley rat study reported body weight loss in dams exposed to 750 mg/kg/day via gavage from GD 12 to postnatal day (PND) 0 (Chen et al. 2010). However, no changes in maternal body weight were observed in several other rodent studies evaluating exposure during gestation and/or lactation at gavage doses ≤1,000 mg/kg/day (Table 2-2).

Drinking water studies in rodents. Wang et al. (2020) exposed Sprague-Dawley rats, Wistar rats, C57BL/6J mice, and BALB/c mice to DEHP in drinking water at doses up to 3,000 mg/kg/day for 30 days. Only Sprague-Dawley rats showed exposure-related changes in body weight, with a significant 16–20% increase in body weight gain during the exposure period at all doses tested (≥300 mg/kg/day).

Dietary studies in rodents. Acute dietary studies do not report body weight effects at doses ≤1,250 mg/kg/day in rodents (Astill et al. 1986; Kitaoka et al. 2013; Sasaki et al. 2003).

In intermediate-duration dietary studies in rats, decreases in body weight or body weight gain >10% in the absence of food consumption changes were reported at doses ranging from 737 to 1,724 mg/kg/day (Agarwal et al. 1986; Gray et al. 1977; Mitchell et al. 1985; Myers 1992b). Body weight changes at dietary doses ranging from 1,114 to 2,496 mg/kg/day were associated with significant reductions in food intake, suggesting potential palatability issues at high doses that may influence body weight due to decreased food consumption (Barber et al. 1987; CMA 1986; Exxon Chemical Americas 1990; Gray et al. 1977; Myers 1992b). However, a paired-feeding study in male rats at 1,440 mg/kg/day indicated that weight loss observed following intermediate-duration exposure could not be completely accounted for based on decreased food intake (Gray et al. 1977).

In intermediate-duration dietary studies in mice, decreases in body weight or body weight gain >10% in the absence of food consumption changes were reported at doses ranging from 1,100 to 7,899 mg/kg/day (Myers 1992b; Toyosawa et al. 2001). Decreased food consumption (18–20%) was only reported in male mice during the first 2 weeks of a 4-week study following exposure to 6,922 mg/kg/day (Myers 1992b). However, this dose was still considered a LOAEL for body weight effects due to the large magnitude of effect (35% decrease in body weight).

In a chronic dietary study in F344 rats, a 15% decrease in body weight in the absence of reduced food intake was observed following exposure to 789 mg/kg/day for 104 weeks (David et al. 2000a). Other 1- to 2-year studies in F344 rats reported reduced body weights only with concomitant reductions in food intake levels at dietary doses ≥322 mg/kg/day (Kluwe et al. 1982a; Marsman et al. 1988; NTP 1982). No dose-related body weight effects were noted in rats at chronic doses up to 300 mg/kg/day (Carpenter et al. 1953; Voss et al. 2005). In mice, chronic exposure to dietary doses ≥799 mg/kg/day, but not ≤672 mg/kg/day, resulted in decreased body weight in the absence of altered food consumption (David et al. 2000b; Kluwe et al. 1982a; NTP 1982). No exposure-related body weight effects were observed in guinea pigs exposed to doses up to 64 mg/kg/day for 1 year (Carpenter et al. 1953).

In a multigeneration study in Sprague-Dawley rats, exposure-related decreases in body weight were observed in F0 and F1 parental animals at dietary doses of 447–659 mg/kg/day without evidence of decreased food consumption (Blystone et al. 2010; NTP 2005). In other 2-generation studies in Wistar rats, exposure-related decreases in body weight and food consumption were observed in F0 and F1 parental animals at dietary doses of 1,040–1,088 mg/kg/day; no body weight or food consumption effects were observed at ≤380 mg/kg/day (Schilling et al. 1999, 2001). No maternal body weight effects were observed in a gestational/lactational study in Wistar rats at dietary doses up to 405 mg/kg/day (Andrade et al. 2006c; Grande et al. 2006). In gestational studies in mice, maternal body weight effects were observed in the absence of decreased food intake at doses ≥191 mg/kg/day, but not ≤170 mg/kg/day (NTP 1988; Shiota and Nishimura 1982; Shiota et al. 1980; Tyl et al. 1988). No changes in parental body weight were observed in a continuous breeding study in mice at dietary doses up to 390 mg/kg/day (Lamb et al. 1987; Morrissey et al. 1988; NTP 1984) or a 1-generation study in mice at dietary doses up to 180.77 mg/kg/day (Tanaka 2002). However, a 1-generation study by Schmidt et al. (2012) reported an approximate 20% increase in body weight and food consumption in parental mice exposed to dietary levels of 0.05–500 mg/kg/day for 8 weeks.

Additional dietary studies lacking food consumption data report decreased body weight following oral exposure to an acute dose of 3,850 mg/kg/day in mice (Muhlenkamp and Gill 1998), intermediate-duration doses of ≥2,100 mg/kg/day in rats or ≥1,300 mg/kg/day in mice (Agarwal et al. 1986; Mitchell et al. 1985; NTP 1982; Sasaki et al. 2003; Short et al. 1987), or chronic-duration doses of ≥140 mg/kg/day in rats (Ganning et al. 1991; Rao et al. 1990; Tamura et al. 1990). The potential contribution of food unpalatability precludes inclusion of body weight findings from these studies in the LSE table.

Other mammalian species. Body weight effects were only noted in ferrets, with a 31% decrease in body weight after exposure to 1,200 mg/kg/day for 14 months (Lake et al. 1976). However, food consumption was not measured in the study by Lake et al. (1976). No body weight effects were noted in monkeys exposed to 2,500 mg/kg/day via gavage for 13 weeks (Kurata et al. 1998). No exposure-related body weight effects were noted in dogs exposed to 56.6 mg/kg/day via capsule for 1 year (Carpenter et al. 1953).

Mechanisms of Obesity. Kim and Park (2014) suggest several mechanisms for DEHP-induced obesity, including activation of peroxisome proliferator activated receptors (PPARs), disruption of thyroid function (which can lead to altered regulation of energy balance and metabolic function), and epigenetic modulation resulting from a suboptimal fetal environment. Support for these mechanisms based on available experimental data included: (1) increased fat accumulation in DEHP-exposed mice expressing human PPARα; (2) promotion of differentiation and lipid accumulation in 3T3-L1 cells (embryonic mouse fibroblasts that differentiate to adipocyte-like cells) by MEHP, a PPARγ agonist; and (3) decreased plasma T4 levels and iodide uptake in rodent thyroid follicular cells exposed to DEHP (which is suggestive of impaired thyroid function that could lead to decreased metabolic function and subsequent weight gain). Studies in 3T3-L1 mouse adipocytes show that incubation with MEHP increased lipid accumulation, browning-like activation, production of reactive oxygen species (ROS) and altered expression of genes related to adipogenesis, adipocyte differentiation, and carbohydrate/glucose uptake (Hsu et al. 2019, 2020; Qi et al. 2019). Adipogenic processes were not demonstrated in human adipocytes exposed to DEHP (Schaedlich et al. 2018).

Wang et al. (2020) proposed that significant alterations in the gut microbiome following oral DEHP exposure may contribute to increased risk of obesity. Following a 30-day oral exposure, Sprague-Dawley rats, showed an increase in bacterial species *Fimicutes* and *Proteobacteria*, which are associated with obesity and diabetes. Sprague-Dawley rats also showed DEHP-associated weight gain. In contrast, Wistar rats and BALB/c and C57BL/6J mice did not have increased *Fimicutes* and *Proteobacteria* and showed normal weight gain.

Summary. Available human epidemiological studies suggest a potential association between DEHP exposure and obesity in adults. However, most of these studies have numerous limitations arising from cross-sectional design and lack of consistent control for potential confounders. The vast majority of animal studies evaluating body weight focus on body weight decreases following exposure to high levels of DEHP. Many high-dose dietary studies reported decreased food intake, indicating that decreased

palatability at high doses may contribute to observed body weight effects. However, a paired-feeding study showed that decreased body weight was not entirely attributable to decreased food intake. In contrast, a limited number of rodent studies reported elevated body weight following oral exposure; additional endpoints from these studies related to metabolic syndrome (increased adipose tissue and serum leptin) are further discussed in Section 2.18 (Other Noncancer).

2.4 RESPIRATORY

Overview. There are few data pertaining to the potential respiratory effects of human exposure to DEHP. Only one animal study evaluated respiratory function following inhalation exposure to DEHP. Several animal studies evaluated lung weight and/or histology following oral or inhalation exposure. Only one study evaluated nasal histology.

Epidemiology Studies. Kolena et al. (2014) observed improved pulmonary function (ratio of forced expiratory volume in 1 second [FEV_1]/forced vital capacity [FVC]) with higher urinary MEHP levels (median 5.94 ng/mL) in a study of 30 community service workers (mean age 46 years) with exposure to DEHP along with other air, liquid, or solid pollutants for an average of 7.9 years (men) and 5.6 years (women) during waste and recycle processing or loading; other DEHP metabolites were not evaluated. Similarly, Kolena et al. (2020) reported improved pulmonary function (FEV_1/FVC) with higher urinary MEHP, MEHHP, MEOHP, and MECPP levels in 32 male firefighters (mean age 38 years) with exposure to DEHP along with other air pollutants. Interpretation of studies with improved pulmonary function is limited by small sample size. No other studies evaluating lung function in workers following inhalation exposure to DEHP were identified.

Findings in general population studies are mixed. In a panel study with repeated urine samples and spirometry tests in 418 Korean adults >60 years old, increased DEHP metabolite (MEHHP and MEOHP) levels in urine were associated with poorer pulmonary function test scores (FEV_1/FVC and forced expiratory flow at 25–75% of FVC [$FEF_{25–75}$]) only in individuals with specific genetic polymorphisms in catalase (CAT), superoxide dismutase (SOD2) and myeloperoxidase (MPO) genes (GC-GC in CAT, TC-TC in SOD2, and Ag-AG in MPO) (Park et al. 2013). The study authors suggested that gene-environment interactions may alter the effect of DEHP exposure on lung function. A slight negative association between pulmonary function and DEHP exposure also occurred in a cross-sectional study of 3,157 subjects (ages 6–49 years) in Canada, in which an interquartile increase in the sum of DEHP metabolites (MEHP, MEHHP, MEOHP) in the urine was associated with an approximate 1% reduction in

lung function (FEV_1, FVC, and FEV_1/FVC), primarily in males and subjects 17–49 years of age (Cakmak et al. 2014). However, no association between lung function measures and MEHP in urine (mean 2.0 ng/mL in women and 3.3 ng/mL in males) was observed in 240 adult participants in NHANES (1988–1994) (Hoppin et al. 2004). In a cohort study, no association was observed between lung function (FEV1, FVC, peak expiratory flow [PEF], or FEV_1/FVC) in 9-year-old children and urinary MEHP levels measured at 2, 5, or 9 years of age (Lin et al. 2018). In a cross-sectional study of asthmatic children, decreased FEV_1 was associated with urinary MEHHP levels; however, this association was no longer apparent when the analysis was adjusted for outdoor environment indicators (particulate matter, temperature, and relative humidity) (Kim et al. 2018e). No associations were observed with FEV_1/FVC, PEF, or $FEF_{25–75}$ and urinary DEHP metabolite levels.

Unusual lung effects, resembling hyaline membrane disease caused by insufficient surfactant production, were observed 4 weeks after birth in three children who were exposed to DEHP in respirator tubes during mechanical ventilation as preterm infants (Roth et al. 1988). These infants initially showed improvements after birth prior to progressive alterations in the lungs, which were not attributable to typical lung damage associated with artificial ventilation (e.g., oxygen toxicity, barotrauma, or bronchopulmonary dysplasia). Although interpretation of these findings is complicated by the preexisting compromised health status of the preterm infants, information provided by the authors indicated that DEHP was released from the walls of the PVC respiratory tubes used by the infants, supporting the potential for exposure.

Animal Studies. Rapid shallow breathing (35% decrease in tidal volume associated with 15% increase in respiratory rate) was observed during lung function analysis of female mice during the last 10 minutes of a 60-minute exposure to DEHP at 19 ppm (Larsen et al. 2007). No alterations in lung function were reported at 2 ppm, and no other respiratory system endpoints were evaluated. No changes in lung weight were observed in female weanling rats exposed to DEHP at concentrations up to 1.6 ppm for 6 hours/day, 5 days/week for 9 weeks (Ma et al. 2006). At 63 ppm, but not ≤3 ppm, increased lung weights accompanied by thickening of the alveolar septa and proliferation of foam cells were observed in male rats exposed for 6 hours/day, 5 days/week for 4 weeks (Klimisch et al. 1991, 1992). These effects were reversible within an 8-week post-exposure period and were not observed at any time point in similarly exposed females. Additionally, no histopathological lesions were observed in the lungs of male or female rats following exposure (Klimisch et al. 1991, 1992).

One study reported an increased incidence (compared with controls) of eosinophilic bodies in nasal cavities of mice exposed to DEHP at dietary doses of 1,100 mg/kg/day for 26 weeks (no other doses tested) (Toyosawa et al. 2001). No other available studies reviewed nasal effects following oral exposure.

No adverse effects on the trachea or lung were reported in any of the oral animal studies reviewed. In intermediate-duration studies, no changes in lung weights and/or lung or trachea histology were observed in monkeys at doses up to 2,500 mg/kg/day (Kurata et al. 1998), rats at doses up to 3,000 mg/kg/day (Gray et al. 1977; Myers 1992b; NTP 1982; Poon et al. 1997), or mice at doses up to 7,899 mg/kg/day (Myers 1992a, NTP 1982; Toyosawa et al. 2001). In chronic-duration studies, no changes in lung weights or histology were observed in dogs at 56.6 mg/kg/day (Carpenter et al. 1953), rats at doses up to 1,600 mg/kg/day (Carpenter et al. 1953; Kluwe et al. 1982a; NTP 1982; Rao et al. 1990; Voss et al. 2005), or mice at doses up to 1,821 mg/kg/day (Kluwe et al. 1982a; NTP 1982). Pulmonary function was not assessed in any of these studies.

In a developmental study, altered lung structure has been reported in rat offspring exposed to DEHP at gavage doses of 750 mg/kg/day from GD 12 to PND 0 or PND 21(Chen et al. 2010). Lung alterations included increased thickness of alveolar septa and less airspace in the lung on PNDs 1 and 21, which was attributed to a significant increase in the proportion of interstitial lung tissue. However, no clinical signs of respiratory distress were observed in pups. No structural changes were observed in the lungs at either age following exposure to maternal doses ≤100 mg/kg/day (Chen et al. 2010). No changes in lung weights were observed in sexually immature monkeys exposed to 500 mg/kg/day for 14 days (Pugh et al. 2000).

A series of studies reported elevated immune responses in the lungs of mice sensitized to OVA following both inhalation and oral exposure to DEHP (Guo et al. 2012; Han et al. 2014a; Larsen et al. 2007; Wang et al. 2018; Yang et al. 2008). These studies are discussed in Section 2.14 (Immunological).

Summary. Available human and animal data do not suggest that the respiratory system is a sensitive target of DEHP toxicity; however, data on respiratory function and potential nasal effects are limited.

2.5 CARDIOVASCULAR

Overview. Available epidemiological studies evaluating cardiovascular effects (that met selection criteria) include cross-sectional and case-control studies of blood pressure and a single cross-sectional

study of subclinical atherosclerosis. Studies examining serum levels of triglycerides and cholesterol are discussed in Section 2.9 (Hepatic). A limited number of animal studies evaluated cardiovascular effects, including blood pressure, heart weight, and heart histology.

Epidemiology Studies. The potential association between DEHP exposure and high blood pressure was evaluated in three pregnancy cohort studies (one evaluated blood pressure in mothers, two evaluated blood pressure in offspring) and seven cross-sectional studies in the general population (Table 2-4). Four of the seven cross-sectional studies (James-Todd et al. 2016a; Shiue and Hristova 2014; Trasande and Attina 2015; Trasande et al. 2013a) used NHANES data and reported associations between DEHP urinary metabolite levels and increased blood pressure. The other three cross-sectional studies (Ko et al. 2019; Lin et al. 2016, 2020) did not observe associations between DEHP exposure and high blood pressure in Taiwan. These cross-sectional studies are limited by inability to establish temporality between exposure and effect, as well as the use of single urine measurements to assess exposure. In the pregnancy cohorts, no associations were observed between DEHP metabolite concentration in maternal urine and maternal blood pressure or pregnancy-induced hypertensive disorders (Werner et al. 2015) or in offspring blood pressure measured at 4–6 years of age (Vafeiadi et al. 2018a). In another cohort, an association between DEHP metabolite concentration in maternal urine and decreased systolic and diastolic blood pressure was observed in 10-year-old female offspring; no association was observed in male offspring (Sol et al. 2020).

One cross-sectional study evaluated the potential association between DEHP exposure and subclinical atherosclerosis in Taiwanese adolescents and young adults aged 12–30 (Lin et al. 2020). A positive association was observed between urinary MEHP levels and carotid intima-media thickness. No association was noted for urinary MEHHP or MEOHP.

Animal Studies. No changes in heart weight or histology were observed in rats following nose-only exposure to DEHP concentrations up to 63 ppm for 4 weeks (5 days/week, 6 hours/day) (Klimisch et al. 1992). No other studies were located regarding cardiovascular effects in animals after inhalation exposure to DEHP.

Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Blood Pressure

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
James-Todd et al. 2016a Cross-sectional, 965 cases of metabolic syndrome (464 men and 501 women) and 1,754 subjects without metabolic syndrome (924 men and 830 women) (age 20–80 years), United States (NHANES)	**BP**	**ΣDEHP** (MEHP, MEHHP, MEOHP)	With metabolic syndrome: GM (95% CI): 0.13 (0.12, 0.15) Without metabolic syndrome: 0.12 (0.10, 0.13)	**All**: ↑ **Men**: ↑ Women:
Ko et al. 2019 Cross-sectional, 435 adults (388 men, 47 women; mean age 32.16 years), Taiwan	High BP (systolic BP ≥130 mm Hg or diastolic BP ≥85 mm Hg)	ΣDEHP	NR	↔
		MEHP	All: 25th–95th percentile: 0.269–2.789 µg/g Cr Men: 0.263–2.800 Women: 0.299–2.551	NR
		MEHHP	All: 0.908–6.045 Men: 0.910–6.013 Women: 0.841–9.648	NR
		MEOHP	All: 0.486–2.603 Men: 0.479–2.636 Women: 0.505–2.509	NR
Lin et al. 2016 Cross-sectional, 793 adult students including 303 with and 486 without elevated BP in childhood (mean age 21.28 years), Taiwan	Systolic BP	MEHP	IQR: 1.7–38.99 µg/g Cr	↔
		MEHHP	15.86–43.16	↔
		MEOHP	10.18–26.56	↔
Lin et al. 2020 Cross-sectional, 792 adolescents and adults (age 12–30 years), Taiwan	Systolic BP	MEHP	GM (95% CI): 6.1 (5.1, 7.3) µg/g Cr	↔
		MEHHP	27.9 (26.1, 30.0)	↔
		MEOHP	17.5 (16.4, 18.5)	↔

Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Blood Pressure

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Shiue and Hristova 2014 Cross-sectional, 20,293 adults (age ≥20 years) including 660 with high blood pressure and 4,578 with normal blood pressure, United States (NHANES)	**BP**	MEHP	Normal BP: Mean (SD): 4.15 (SD) 16.49 ng/mL High BP: 3.36 (6.62)	↔
		MEHHP	Normal BP: 27.75 (155.35) High BP: 25.03 (50.74)	↑
		MEOHP	Normal BP: 16.45 (97.03) High BP: 15.22 (25.48)	↑
		MECPP	Normal BP: 40.10 (249.63) High BP: 38.52 (64.13)	↑
		Shiue (2015a, 2015b) evaluated associations between blood pressure and urinary metabolite levels in subsets of this population (2009–2010 and 2011–2012 NHANES participants, respectively). In these studies, associations were seen with the same urinary metabolites.		
Sol et al. 2020 Cohort, 1,064 mother-child pairs (538 boys, 526 girls; median age 9.7 years), Netherlands	Systolic and diastolic BP in boys	ΣDEHP (MECPP, MEHHP, MEOHP, MCMHP)	Maternal IQR (1st trimester): 90.0–328.6	↔
			2nd trimester: 54.1–184.2	↔
			3rd trimester: 74.7–227.5	↔
	Systolic and diastolic BP in girls	**ΣDEHP**	1st trimester: 88.8–298.6	↔
			2nd trimester: 48.7–174.3	↔
			3rd trimester: 81.4–272.8	↓

Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Blood Pressure

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Trasande and Attina 2015 Cross-sectional, 1,329 children (age 8–19 years), United States (NHANES)	**Diastolic or systolic BP**	**ΣDEHP** (MEHP, MEHHP, MEOHP, MECPP)	IQR: 0.077–0.313 µM	↑
	BP >90th percentile for age/height z-score/sex	ΣDEHP	See above	↔
Trasande et al. 2013a Cross-sectional, 2,463 children and adolescents (age 6–19 years), United States (NHANES)	**Systolic BP**	**ΣDEHP** (MEHP, MEHHP, MEOHP, MECPP)	IQR: 0.166–0.704 M	↑
	Diastolic BP	ΣDEHP	See above	↔
	BP >90th percentile for age/height z-score/sex	ΣDEHP	See above	↔
Vafeiadi et al. 2018a Cohort, 260 mothers and their 500 children (279 boys, 221 girls; age 4–6 years), Greece	Systolic and diastolic BP	ΣDEHP (MEHP, MEHHP, MEOHP)	Maternal IQR (1st trimester): 0.1–0.2 µmol/g	↔
			Child: 0.2–0.5	↔
Werner et al. 2015 Cohort, 369 pregnant women (age ≥18 years), United States (Ohio)	Diastolic BP, systolic BP, or pregnancy-induced hypertensive disorder	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	Average concentration from 16 and 26 weeks of gestation: 53–159 µg/g Cr	↔

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; BP = blood pressure; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; IQR = interquartile range; MCMHP = mono-[(2-carboxymethyl)-hexyl] phthalate; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NHANES = National Health and Nutrition Examination Survey; SD = standard deviation

There is limited evidence of altered cardiac function in mice following intermediate-duration oral exposure to DEHP. Two studies in mice reported elevated blood pressure after exposure to ≥0.1 mg/kg/day for 42–45 days (Deng et al. 2019; Xie et al. 2019). Deng et al. (2019) also reported elevated heart rate at 1 mg/kg/day (heart rate not assessed by Xie et al. 2019). These findings were accompanied by a significant thickening of the ventricular and aortic walls. Biochemical findings suggest that elevated blood pressure occurred due to activation of angiotensin converting enzyme (ACE), which inhibits the bradykinin-nitric oxide pathway. Another study reported elevated heart rate and mean blood pressure in mice orally exposed to 180 mg/kg/day for 3 weeks (Ding et al. 2019). Serum levels of cardiac troponin 1 and hypersensitive C-reactive protein were elevated at 18 and 180 mg/kg/day, respectively.

No changes in heart weight were observed; hearts were not examined for histopathological changes. Li et al. (2018) reported elevated relative heart weight and increased lipid droplets in cardiac papillary muscle cells in mice following a 35-day gavage exposure to ≥1 and ≥100 mg/kg/day, respectively. Cardiac function was not tested in this study, but metabonomic, gene expression, and enzyme activity analysis revealed that DEHP altered endogenous metabolites and metabolic pathways involved in fatty acid and glucose metabolism in cardiomyocytes at all doses.

Additional studies have reported elevated blood pressure in rodents following intermediate- or chronic-duration oral exposure to DEHP; however, findings were considered secondary to observed renal dysfunction, as discussed in Section 2.10 (Renal). Kamijo et al. (2007) reported elevated systolic blood pressure (compared with controls) in mice exposed to approximately 9.5 or 48.5 mg/kg/day of DEHP in feed for 6–22 months. Wei et al. (2012) reported elevated blood pressure associated in adult offspring of maternal rats exposed to DEHP from GD 0 to PND 21 at 0.25 or 6.25 mg/kg/day; systolic pressure was elevated in low dose males on day 21, systolic pressure was elevated in both sexes at both doses at 33 weeks, and diastolic pressure was elevated in both sexes at the low dose at 33 weeks. In contrast, a mild (but statistically significant) 4% decrease in systolic blood pressure was observed in adult offspring of rats exposed to 300 mg/kg/day from GD 14 to PND 0; neither kidney function nor kidney histology were evaluated in adult offspring in this study (Martinez-Arguelles et al. 2013).

In the majority of other oral studies reviewed, no changes in heart weight or histology were observed; however, cardiovascular function was not assessed in any of these studies. No changes in heart weight were observed in sexually immature monkeys exposed to 500 mg/kg/day for 14 days (Pugh et al. 2000). In intermediate-duration studies, no changes in heart weight and/or histology were observed in monkeys at doses up to 2,500 mg/kg/day (Kurata et al. 1998), rats at doses up to 10,000 mg/kg/day (Dalgaard et al.

2000; Gray et al. 1977; Myers 1992b; NTP 1982; Poon et al. 1997; Shaffer et al. 1945), or mice at doses up to 7,899 mg/kg/day (Myers 1992a; NTP 1982; Toyosawa et al. 2001). However, relative heart weights were significantly decreased in rat dams following exposure to 300 mg/kg/day from GD 8 to PND 21 (Nardelli et al. 2017). In chronic-duration studies, no changes in heart weight or histology were observed in dogs at 56.6 mg/kg/day (Carpenter et al. 1953), ferrets at 1,200 mg/kg/day (Lake et al. 1976), rats at doses up to 1,600 mg/kg/day (Carpenter et al. 1953; Kluwe et al. 1982a; NTP 1982), or mice at doses up to 1,821 mg/kg/day (Kluwe et al. 1982a; NTP 1982).

Potential effects on human heart muscle contractility and rhythm were identified in *in vitro* and *ex vivo* studies. MEHP displayed a dose-dependent negative inotropic effect that weakened human atrial trabecular contractions at concentrations of 15–200 µg/mL, with an IC_{50} of 85 µg/mL (Barry et al. 1990). Exposure to MEHP produced electrophysiological changes in the isolated rat heart (increased action potential triangulation, altered action potential duration restitution curve), suggesting an increased risk of arrhythmia (Jaimes et al. 2019). This suggests the possibility that high levels of serum MEHP could have a cardiotoxic effect in humans. However, rapid metabolism of MEHP would act to minimize the probability that MEHP concentrations would reach the concentration associated with the negative inotropic effect. The authors suggested that infants with multisystem failures would be the group at greatest risk to a cardiotoxic effect of MEHP. Yet, there was no indication of cardiovascular effects in 18 infants who had increased plasma levels of DEHP (8.3±5.7 µg/mL, mean highest concentration) from exposure during ECMO therapy for 3–10 days (DEHP had leached from plastic tubing) (Karle et al. 1997). Cardiac performance was evaluated by using echocardiograms to estimate output from heart rate, systolic blood pressure, left ventricular shortening fraction, and stroke volume measurements.

Summary. Mixed results were obtained in human studies for the association between DEHP exposure and elevated blood pressure. In general, available animal data do not indicate that the cardiovascular system is a sensitive target of DEHP toxicity. Evidence from animal studies suggests that altered blood pressure is likely secondary to renal toxicity following exposure to DEHP; however, one study (Xie et al. 2019) reported elevated blood pressure at doses below those associated with renal damage.

2.6 GASTROINTESTINAL

Human Studies. Wang et al. (2015) reported increased rates of nausea and vomiting in Chinese workers exposed to DEHP at three different PVC manufacturing facilities (average exposures ranging between 233 and 707 µg/m^3 DEHP in the three factories). These effects may be secondary to neurotoxicity (see

Section 2.15), rather than a direct effect on the gastrointestinal symptom. No other studies were located regarding gastrointestinal effects in humans after inhalation exposure to DEHP.

Acute exposures to large oral doses of DEHP can cause gastrointestinal distress. When two adult male volunteers ingested a single oral dose of 5 or 10 g DEHP (70 and 140 mg/kg based on 70-kg body mass), the individual consuming the larger dose complained of mild abdominal pain and diarrhea; no other effects of exposure were noted (Shaffer et al. 1945).

Animal Studies. No studies were located regarding gastrointestinal effects in animals after inhalation exposure to DEHP.

In oral studies, pseudoductular lesions or altered acinar cell foci were observed in the pancreas of rats administered dietary DEHP at 1,600 mg/kg/day for 108 weeks (only dose tested) (Rao et al. 1990). These lesions are expected to affect digestive system functions of the pancreas, as opposed to endocrine function. No other chronic-duration studies reported histopathological lesions in the gastrointestinal system for dogs given 56.6 mg/kg/day (Carpenter et al. 1953), rats at doses up to 939 mg/kg/day (Carpenter et al. 1953; David et al. 2000a; Kluwe et al. 1982a; NTP 1982), or mice at doses up to 1,821 mg/kg/day (David et al. 2000b; Kluwe et al. 1982a; NTP 1982). Similarly, no histopathological lesions in the gastrointestinal system were observed following intermediate-duration exposure to doses up to 2,500 mg/kg/day in monkeys (Kurata et al. 1998), 3,000 mg/kg/day in rats (Gray et al. 1977; Myers 1992b; NTP 1982; Poon et al. 1997; Wang et al. 2020), or 7,899 mg/kg/day in mice (Myers 1992a; NTP 1982; Toyosawa et al. 2001; Wang et al. 2020).

Summary. The dataset is too limited to evaluate potential gastrointestinal effects from DEHP exposure.

2.7 HEMATOLOGICAL

Epidemiological Studies. Wang et al. (2015) reported no differences in hemoglobin levels between 352 DEHP-exposed Chinese workers in three PVC factories (factory average exposures ranging from 233 to 707 µg/m^3 DEHP) and 104 unexposed workers (average exposure, 0.26 µg/m^3 DEHP). No other studies examining hematological effects in humans after exposure to DEHP were located.

Animal Studies. No changes were observed in a comprehensive hematological evaluation in rats following nose-only exposure to DEHP concentrations up to 63 ppm for 4 weeks (5 days/week,

6 hours/day) (Klimisch et al. 1992). No other studies were located regarding hematological effects in animals after inhalation exposure to DEHP.

In nonhuman primates, no hematological changes were reported following oral DEHP exposure. Exposure to DEHP for 14–28 consecutive days did not cause hematological changes in sexually immature or mature Cynomolgus monkeys at doses of 500 or 1,000 mg/kg/day, respectively (Pugh et al. 2000; Satake et al. 2010) or marmoset monkeys at 2,000 mg/kg/day (ICI Americas Inc. 1982; Rhodes et al. 1986). Similarly, no adverse hematological effects were reported in marmoset monkeys following exposure to DEHP at doses up to 2,500 mg/kg/day via gavage for 13 weeks (Kurata et al. 1998).

Altered hematological parameters have been inconsistently reported in rodents following oral exposure to DEHP. Where effects were reported, sex-related differences were generally observed with increased sensitivity in males. Slight but significant decreases in red blood cell counts and serum hemoglobin were seen in male Sprague-Dawley rats exposed to dietary DEHP at approximately 375.2 mg/kg/day for 13 weeks; doses ≤37.6 mg DEHP/kg/day were without hematological effect in males (Poon et al. 1997). No hematological effects were noted in similarly exposed female rats at doses up to 419.3 mg/kg/day (Poon et al. 1997). In another 13-week dietary study in F344 rats, significant reductions in red blood cell count, hemoglobin, and hematocrit, and an increase in platelets, were observed in males at ≥850.1 mg/kg/day and significant reductions in hemoglobin, hematocrit, myeloid: erythroid ratio, and segmented neutrophils were observed in females at 1,857.6 mg/kg/day; no biologically significant hematological changes were observed in males or females at ≤261.2 or 918.4 mg/kg/day, respectively (Myers 1992b). Additionally, in a 17-week dietary study in Sprague-Dawley rats, significantly reduced hemoglobin levels were observed in males and significantly reduced packed cell volume was observed in both males and females at ≥737 mg/kg/day, but not ≤154 mg/kg/day (Gray et al. 1977). However, exposure of male albino rats to doses of 200–1,900 mg/kg/day DEHP in the diet for 90 days had no effect upon red blood cell counts, hemoglobin levels, or differential white cell counts (Shaffer et al. 1945). No changes in comprehensive hematological evaluations were observed in Sprague-Dawley rats exposed to gavage doses up to 150 mg/kg/day on PNDs 6–96 (Kim et al. 2018c). In 28-day studies in mice, significantly reduced hemoglobin and hematocrit were observed in males and females at doses ≥1,209 and 2,888 mg/kg/day, respectively; no hematological changes were observed at oral doses ≤400 mg/kg/day (Myers 1992a; Xu et al. 2019). No changes have been observed in comprehensive hematological evaluations in chronic-duration studies at dietary doses up to 939 mg/kg/day in rats or 1,458 mg/kg/day in mice (Carpenter et al. 1953; David et al. 2000a, 2000b).

Summary. Data are sparse, but it does not appear that the primate hematological system is sensitive to DEHP exposure. Inconsistent hematological effects are reported in rodents exposed to DEHP; where effects were observed, male rats were generally more sensitive than female rats.

2.8 MUSCULOSKELETAL

Human Studies. In a cohort study of 481 mother-child pairs, maternal urinary DEHP metabolite levels were associated with decreased skeletal muscle index (SMI) in 6-year-old girls, but not boys (Lee et al. 2020). No associations were observed between maternal urinary DEHP metabolite levels and percentage of skeletal muscle (% SM) or between child urinary DEHP metabolite levels and SMI or % SM. No additional studies were located regarding musculoskeletal effects in humans after exposure to DEHP.

Animal Studies. No changes were observed in the histology of the gastrocnemius muscles of rats following nose-only exposure to DEHP concentrations up to 63 ppm for 4 weeks (5 days/week, 6 hours/day) (Klimisch et al. 1992). No other studies were located regarding musculoskeletal effects in animals after inhalation exposure to DEHP.

No adverse effects on the musculoskeletal system were reported in an intermediate-duration study in marmoset monkeys at doses up to 2,500 mg/kg/day (Kurata et al. 1998).

One gavage study reported decreased bone mineral density and bone volume fraction coupled with reduced osteoblastogenesis and mineralization of bone marrow stromal cells in ICR mice exposed to ≥10 mg/kg/day for 8 weeks (Chiu et al. 2018c). Osteoblastogenesis was replaced by adipogenesis in bone marrow stromal cells. At 100 mg/kg/day, trabecular bone thickness and cell number were also reduced. No changes in cortical bone thickness or trabecular separation were observed. In other rodent studies, no adverse musculoskeletal effects were reported in acute-, intermediate-, or chronic-duration oral studies in rats at doses up to 1,100, 3,000, or 939 mg/kg/day, respectively (Astill et al. 1986; David et al. 2000a; Gray et al. 1977; Kluwe et al. 1982a, 1982b, 1985; Myers 1992b; NTP 1982; Poon et al. 1997); or in intermediate- or chronic-duration studies in mice at doses up to 2,600 or 1,821 mg/kg/day, respectively (David et al. 2000b; Kluwe et al. 1982a, 1982b, 1985; NTP 1982; Toyosawa et al. 2001).

Summary. Epidemiological data for DEHP exposure and the musculoskeletal endpoint are limited to a single cohort study of mother-child pairs. Maternal urinary levels of DEHP were associated with a decrease in the SMI in 6-year-old girls, but not boys, and no association was found for child urinary levels

(Lee et al. 2020). An adult monkey study and most rodent studies indicate that the musculoskeletal system is not adversely affected from DEHP exposure. One mouse study (Chiu et al. 2018a, 2018b) indicated altered trabecular (but not cortical) bone density, volume, and thickness following exposure to DEHP.

2.9 HEPATIC

Overview. Human data on hepatic effects of DEHP are limited to evaluation of clinical chemistry parameters, including serum enzymes and lipid and cholesterol evaluation. Numerous oral and inhalation animal studies have evaluated hepatic effects following exposure to DEHP, including serum chemistry, biochemistry in liver tissue, liver weight, and liver histology. Several secondary sources have reviewed potential mechanisms of DEHP hepatotoxicity.

Epidemiology Studies. Wang et al. (2015) observed increases in facility-averaged serum alanine transaminase (ALT) (2.4–3-fold higher) and gamma-glutamyl transferase (GGT) (1.4–1.6-fold higher) in 352 Chinese workers exposed to DEHP at three different PVC manufacturing facilities (facility average exposures ranging between 233 and 707 μg/m^3 DEHP in the 3 factories) when compared with levels in 104 unexposed workers (average exposure, 0.26 μg/m^3 DEHP). Plasma cholinesterase activity was reduced by >30% in post-exposure samples of some workers at these facilities (25, 10, and 7 workers from small-, medium-, and large-sized facilities, respectively). This enzyme is synthesized by the liver; therefore, a reduction in plasma cholinesterase activity may be indicative of liver dysfunction (Meng et al. 2013). A correlation was observed between reduced plasma cholinesterase activity and DEHP residues in plasma (Wang et al. 2015). Serum levels of total bilirubin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total protein did not differ among the groups. Serum liver enzymes (ALT and AST) were not increased in 52 Taiwanese children exposed to DEHP in contaminated foods (dose estimates ranged up to 0.1874 mg/kg/day; Wu et al. 2013); however, the duration of exposure was not known.

Epidemiological studies that examined serum cholesterol and triglycerides and used urinary metabolite levels to assess exposure are shown in Table 2-5. A positive association between hypertriglyceridemia and DEHP exposure was reported in a cross-sectional study of NHANES participants with and without metabolic syndrome (data from cases and non-cases were combined for regression analysis; James-Todd et al. 2016a), in a cohort study of 3–5- and 7–9-year-old children (Han et al. 2019), and in cord blood in

Table 2-5. Selected Epidemiological Studies of DEHP Exposure and Serum Lipid/Cholesterol Alterations

Reference, study type, and population	Outcome evaluated	Metabolite[a]	Urine concentration	Result
Han et al. 2019 Cohort, 164 children (assessed at age 3–5 and 7–9 years), South Korea	**Triglycerides**	**ΣDEHP**	3–5 years: IQR: 258.18–595.69 µg/g Cr	↔
			7–9 years: 159.43–370.86	↑
		MEHP	3–5 years: 14.14–37.55	↔
			7–9 years: 10.35–31.76	↑
		MEHHP	3–5 years: 89.79–212.80	↔
			7–9 years: 58.19–127.45	↑
		MEOHP	**3–5 years: 54.92–134.51**	↑
			7–9 years: 33.33–74.17	↑
		MECPP	3–5 years: 75.08–190.57	↔
			7–9 years: 49.22–120.65	↑
	HDL cholesterol	**ΣDEHP**	**3–5 years**: see above	↓
			7–9 years: see above	↔
		MEHP, MEHHP, MEOHP, MECPP	3–5 years: see above	↔
			7–9 years: see above	↔
James-Todd et al. 2016a Cross-sectional, 965 cases of metabolic syndrome and 1,754 controls without metabolic syndrome (age 20–80 years), United States (NHANES)	**Triglycerides**	**ΣDEHP** (MEHP, MEHHP, MEOHP)	Cases: GM (95% CI): 0.13 (0.12, 0.15) ng/mL Controls: 0.12 (0.10, 0.13)	↑
	Low HDL cholesterol	ΣDEHP	See above	↔
Kim et al. 2016a Cohort, 128 infants, Korea	**BMI or triglyceride in cord blood**	**ΣDEHP**	NR	↑
		MEHHP	Infant (first urine) IQR: 3.21–11.87 ng/mL	↑
		MEOHP	1.51–6.50	↑
	Total cholesterol in cord blood	ΣDEHP, MEHHP, or MEOHP	See above	↔

Table 2-5. Selected Epidemiological Studies of DEHP Exposure and Serum Lipid/Cholesterol Alterations

Reference, study type, and population	Outcome evaluated	Metabolite[a]	Urine concentration	Result
Ko et al. 2019 Cross-sectional, 435 adults (388 men, 47 women; mean age 32.16 years), Taiwan	High triglycerides (≥150 mg/dL) or low HDL (male <40 mg/dL, female <50 mg/dL)	ΣDEHP	NR	↔
		MEHP	All: 25th–95th percentile: 0.269–2.789 µg/g Cr Men: 0.263–2.800 Women: 0.299–2.551	NR
		MEHHP	All: 0.908–6.045 Men: 0.910–6.013 Women: 0.841–9.648	NR
		MEOHP	All: 0.486–2.603 Men: 0.479–2.636 Women: 0.505–2.509	NR
Lin et al. 2020 Cross-sectional, 792 adolescents and adults (age 12–30 years), Taiwan	**HDL cholesterol**	**MEHP**	GM (95% CI): 6.1 (5.1, 7.3) µg/g Cr	↓
		MEHHP	27.9 (26.1, 30.0)	↔
		MEOHP	17.5 (16.4, 18.5)	↔
	Triglycerides or LDL cholesterol	MEHP, MEHHP, MEOHP	See above	↔
Lin et al. 2016 Cross-sectional, 793 students including 303 with and 486 without elevated blood pressure in childhood, (mean age 21.28 years), Taiwan	**HDL cholesterol**	**MEHP**	IQR: 1.7–38.99 µg/g Cr	↑
		MEHHP	15.86–43.16	↔
		MEOHP	10.18–26.56	↔
	Triglycerides or LDL cholesterol	MEHP, MEHHP, MEOHP	See above	↔

Table 2-5. Selected Epidemiological Studies of DEHP Exposure and Serum Lipid/Cholesterol Alterations

Reference, study type, and population	Outcome evaluated	Metabolite[a]	Urine concentration	Result
Perng et al. 2017 Cohort, 240 mother-adolescent pairs (112 boys, 128 girls; age 8–14 years), participants in the Early Life Exposure in Mexico to Environmental Toxicants Project, Mexico	**LDL cholesterol**	**ΣDEHP** (MEHP, MEHHP, MEOHP, MECPP)	Maternal: Mean (SD): 0.3 (0.3 nmol/mL)	↔
			Child (boys): 1.7 (11.0)	↔
			Child (girls): 0.6 (0.6)	↓
	Total, or HDL cholesterol or triglycerides	ΣDEHP	Maternal: see above	↔
			Child (boys): see above	↔
			Child (girls): see above	↔
Trasande and Attina 2015 Cross-sectional, 1,329 children (age 6–19 years), United States (NHANES)	Triglycerides or HDL cholesterol	ΣDEHP	IQR: 0.077–0.313 µM	↔
Trasande et al. 2013a Cross-sectional, 2,463 children and adolescents (age 6–19 years), United States (NHANES)	Triglycerides or HDL cholesterol	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	IQR: 0.166–0.704 mol/L	↔
Vafeiadi et al. 2018a Cohort, 260 mothers and their 500 children (279 boys, 221 girls; age 4–6 years), Greece	**Total cholesterol**	**ΣDEHP** (MEHP, MEHHP, MEOHP)	Maternal IQR (1st trimester): 0.1–0.2 µmol/g	↔
			Child: 0.2–0.5	All: ↔ Boys: ↔ **Girls:** ↑
	HDL cholesterol	ΣDEHP	Maternal: see above	↔
			Child: see above	↔

Table 2-5. Selected Epidemiological Studies of DEHP Exposure and Serum Lipid/Cholesterol Alterations

Reference, study type, and population	Outcome evaluated	Metabolite[a]	Urine concentration	Result
Yaghjyan et al. 2015a, 2015b Cross-sectional, 6,005 women (age ≥18 years), United States (NHANES)	Triglycerides or total, HDL, or LDL cholesterol	ΣDEHP	IQR: 19.59–58.66 µg/g Cr	↔
		MEHP	1.49–5.95	↔
		MEHHP	9.86–31.09	↔
		MEOHP	6.83–19.84	↔
		MECPP	17.16–49.78	↔

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; BMI = body mass index; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; HDL = high-density lipoprotein; IQR = interquartile range; LDL = low-density lipoprotein; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NHANES = National Health and Nutrition Examination Survey; NR = not reported

an infant cohort (Kim et al. 2016a). However, no association between DEHP exposure and triglyceride levels were observed in another cohort (Perng et al. 2017) or in cross-sectional studies (Ko et al. 2019; Lin et al. 2016, 2020; Trasande and Attina 2015, Trasande et al. 2013a; Yaghjyan et al. 2015a, 2015b). A negative relationship between MEHP (but not MEHHP or MEOHP) in urine and high-density lipoprotein (HDL) cholesterol levels was observed in cross-sectional studies of young adults in Taiwan (Lin et al. 2020) and between the sum of DEHP metabolites and HDL levels in children at 3–5 years of age (but not 7–9 years of age) in a South Korean cohort (Han et al. 2019). However, no association was observed in additional studies of this endpoint (James-Todd et al. 2016a; Ko et al. 2019; Perng et al. 2017; Trasande and Attina 2015; Trasande et al. 2013a; Vafeiadi et al. 2018a; Yaghjyan et al. 2015a, 2015b). In a mother-child cohort, Vafeiadi et al. (2018a) observed increased total cholesterol in 4–6-year-old girls with increased urinary metabolites in girls (but not maternal levels); no changes were observed in boys. Total cholesterol in cord blood was not associated with maternal urinary DEHP metabolites (Kim et al. 2016a). Perng et al. (2017) observed decreased low-density lipoprotein (LDL) in 8–14-year-old girls with increased urinary metabolites in girls (but not maternal levels); no changes were observed in boys for LDL or in either sex for total cholesterol.

Available cross-sectional studies did not indicate an association between DEHP urinary metabolite levels and LDL (Yaghjyan et al. 2015a, 2015b) or total cholesterol levels (Lin et al. 2016, 2020; Yaghjyan et al. 2015a, 2015b).

Animal Studies—Histopathology and Morphology. In the only inhalation study that evaluated liver histology, no exposure-related hepatic lesions were observed in rats following intermittent exposure to concentrations up to 63 ppm for 4 weeks (Klimisch et al. 1991, 1992).

In oral studies in nonhuman primates, no histopathological changes were observed in monkeys exposed to doses up to 2,500 mg/kg/day for up to 13 weeks (ICI Americas Inc. 1982; Kurata et al. 1998; Rhodes et al. 1986; Satake et al. 2010; Short et al. 1987).

Other than observations of hepatocellular hypertrophy (described below with liver weight data), most oral studies in rodents (Table 2-2) did not find exposure-related changes during microscopic examination of the liver following exposure to DEHP at acute doses up to 1,500 mg/kg/day or intermediate doses up to 10,000 mg/kg/day. Additionally, no histopathological changes were observed in hamsters exposed to doses up to 1,000 mg/kg/day for 14 days (Lake et al. 1984).

A few intermediate-duration studies have reported exposure-related hepatic lesions other than hepatocellular hypertrophy in rats following oral DEHP exposure. Centrilobular necrosis and inflammation were observed in F344 female rats after exposure to 1,500 mg/kg/day for 14 days, but not at doses ≤500 mg/kg/day (Berman et al. 1995). Another study in F344 rats reported marked individual cell necrosis with a ductal cell reaction in one lobe of the liver in 1/5 males following dietary exposure to 105 mg/kg/day for 21 days; however, these lesions were not observed in males exposed to higher doses (667–2,101 mg/kg/day) or females at doses up to 1,892 mg/kg/day (CMA 1986). Because this finding was limited to a single animal at a low dose only, it is likely an incidental effect. In a 28-day study in male F344 rats, an increased incidence of hepatocyte cytoplasmic eosinophilia was observed at 2,496 mg/kg/day, but not ≤1,093 mg/kg/day (Exxon Chemical Americas 1990). Increased incidence of hepatocellular eosinophilia was also observed in adult F1 rats in a 2-generation study in Wistar rats at DEHP doses ≥340 mg/kg/day, but not 113 mg/kg/day (Schilling et al. 2001). Additional lesions at 1,088 mg/kg/day in F1 adults included focal bile duct proliferation and altered hepatic foci. However, these hepatic lesions were not observed in another 2-generation study in Wistar rats at dietary doses up to approximately 1,040 mg/kg/day (Schilling et al. 1999). Other studies in Wistar rats reported increased incidence of congestion, mononuclear cell infiltration, and sinusoidal degeneration following exposure to ≥100 mg/kg/day via gavage for 4 weeks (Aydemir et al. 2018), slight centrilobular steatosis following exposure to ≥1,000 mg/kg/day via drinking water for 30 days (Wang et al. 2020), and disordered hepatocyte cords and vacuolar degeneration at ≥5 mg/kg/day via gavage for 8 weeks (Zhang et al. 2019, 2020c). One study in Sprague-Dawley rats qualitatively reported vacuolar degeneration and inflammatory infiltration after exposure to ≥0.05 mg/kg/day for 15 weeks, which progressed to central necrosis at 500 mg/kg/day (Zhang et al. 2017). Effects reported in other intermediate-duration Sprague-Dawley rat studies were observed at much higher oral doses, including vacuolation, hepatic sinusoidal dilation, and reduction in hepatocyte number following exposure to ≥500 mg/kg/day for 30 days (Ye et al. 2017) and liver steatosis at ≥1,000 mg/kg/day for 30 days (Wang et al. 2020).

Similarly, a few intermediate-duration studies have reported exposure-related hepatic lesions other than hepatocellular hypertrophy in mice following oral DEHP exposure. In a 30-day drinking water study, liver steatosis was observed in BALB/c mice at 3,000 mg/kg/day, and mild inflammatory cell infiltrates were observed in C57BL/6J mice at ≥300 mg/kg/day (Wang et al. 2020). In a dietary study in mice, moderate focal coagulative necrosis was observed in the livers of B6C3F1 mice at doses ≥1,209 mg/kg/day for 13 weeks, but not at doses of approximately 245–270 mg/kg/day (Myers 1992a).

In chronic studies in F344 rats, observed hepatic lesions other than hepatocellular hypertrophy included spongiosis hepatis (cystic degeneration) in males at ≥147 mg/kg/day, increased incidence of clear cell foci in males at ≥320 mg/kg/day, and increased cytoplasmic eosinophilia and Kupffer cells in males and females at 789 and 939 mg/kg/day, respectively (David et al. 2000a; Kluwe et al. 1982a, NTP 1982). David et al. (1999, 2000b) also reported increased cytoplasmic eosinophilia in male and female B6C3F1 mice exposed to 1,266 or 1,458 mg/kg/day, respectively, but not at doses up to 354.2 mg/kg/day. However, no histopathological changes in the liver were observed in another 2-year study in mice at doses up to 1,821 mg/kg/day (Kluwe et al. 1982a; NTP 1982). Other chronic studies in rats did not report hepatic lesions at doses up to 300 mg/kg/day (Carpenter et al. 1953; Voss et al. 2005). In other species, exposure-related hepatic lesions were not observed in guinea pigs at doses up to 64 mg/kg/day or dogs exposed to 56.6 mg/kg/day for 1 year (Carpenter et al. 1953).

Morphological examinations have shown enlarged liver cells and lipofuscin deposits in rats exposed to DEHP, indicating that peroxidation of cellular lipids had occurred (Lake et al. 1987; Mitchell et al. 1985; Price et al. 1987). On a microscopic level, there was a definite increase in hepatic peroxisomes in the centrilobular and periportal areas of the liver and there was often an increase in the number of mitochondria (Hodgson 1987; Nair and Kurup 1987a). Lipid filled lysosomes were observed in some cases (Mitchell et al. 1985). Each of these changes contributed to cellular hypertrophy. Many of the morphological changes described above were seen in the male rats at doses ≥50 mg/kg/day but did not appear in the females until doses ≥200 mg/kg/day (Mitchell et al. 1985), indicating that male rats are somewhat more susceptible than females.

Two studies (Arcadi et al. 1998; Maranghi et al. 2010) indicated histopathological changes in developing animals; these studies are discussed in Section 2.17 (Developmental).

Animal Studies—Clinical Chemistry. In the only inhalation study that evaluated hepatic serum enzymes, no exposure-related changes were observed in rats following intermittent exposure to concentrations up to 63 ppm for 4 weeks (Klimisch et al. 1991, 1992).

In monkeys, no changes in hepatic serum enzyme levels were observed at doses up to 2,500 mg/kg/day for up to 13 weeks (ICI Americas Inc. 1982; Kurata et al. 1998; Rhodes et al. 1986). Small, but significant, increases in serum ALT and AST were observed in Wistar rats following exposure to ≥100 mg/kg/day for 4 weeks; larger increases were observed for ALT and AST (1.8- and 2.4-fold, respectively) were observed only at 400 mg/kg/day (Aydemir et al. 2018). Another study in Wistar rats

reported significant increases in serum ALT (~20–25%) following exposure to 300 or 1,000 mg/kg/day, but not at 3,000 mg/kg/day (Wang et al. 2020). In similarly exposed Sprague-Dawley rats, serum ALT, ALP, and AST were significantly increased by approximately 20–30% at 3,000 mg/kg/day (Wang et al. 2020). However, a 15-week study in Sprague-Dawley rats reported effects at much lower doses, with a 120–145% increase in serum ALP at doses ≥5 mg/kg/day and a 70–100% increase in serum AST and ALT at 500 mg/kg/day (Zhang et al. 2017). In other rat studies, no biologically relevant changes in hepatic serum enzyme levels have been reported following acute- or intermediate-duration oral exposure up to 1,858 mg/kg/day (Astill et al. 1986; Kim et al. 2018c; Myers 1992b; Nardelli et al. 2017; Poon et al. 1997) or chronic-duration oral exposure up to 939 mg/kg/day (David et al. 2000a). In C57BL/6J mice, a 35-day gavage study reported a 46–83% increase in serum ALT at ≥1 mg/kg/day (Li et al. 2018). In contrast, no exposure-related changes in serum ALT, AST, or ALP were observed in C57BL/6J mice following drinking water exposure to doses up to 3,000 ppm for 30 days (Wang et al. 2020). In similarly exposed BALB/c mice, serum ALP was significantly increased by approximately 30–40% following drinking water exposure to ≥1,000 mg/kg/day for 30 days (Wang et al. 2020). In ICR mice, a significant 23% increase in serum ALP and 54% increase in serum AST was observed following oral exposure to 180 mg/kg/day for 3 weeks, but not ≤18 mg/kg/day (Ding et al. 2019). In other mouse studies, no changes in hepatic serum enzyme levels were observed following intermediate-duration oral exposure up to 7,899 mg/kg/day (Myers 1992a) or chronic-duration exposure up to 1,458 mg/kg/day (David et al. 2000b).

Decreases in circulating cholesterol and/or triglyceride levels were seen in rats exposed to DEHP at doses >100 mg/kg/day (Astill et al. 1986; Barber et al. 1987; CMA 1986; Poon et al. 1997; Reddy et al. 1976; Wang et al. 2020). DEHP also inhibited cholesterol synthesis in the liver from male rats and rabbits (Bell 1982). In a subsequent study, Bell and Buthala (1983) demonstrated that the inhibition of cholesterol synthesis in the liver was due to a reduction in the activity of microsomal acylCoA:cholesterol acyltransferase, an enzyme responsible for the esterification of cholesterol. The lowered serum cholesterol concentration may also be due to the inhibition of cholesterol synthesis and stimulation of the conversion of cholesterol to bile acids in the liver (Nair and Kurup 1986). In contrast, increased cholesterol and/or triglycerides were reported in rats exposed to 500 mg/kg/day for 8 weeks (Zhang et al. 2019, 2020c; Zhou et al. 2019) and mice exposed to ≥1.8 mg/kg/day for 3 weeks or ≥1 mg/kg/day for 35 days (Ding et al. 2019; Li et al. 2018). Ding et al. (2019) suggested that this finding was due to altered lipid metabolism associated with decreased hepatic lipase and lecithin-cholesterol acyltransferase levels. In a drinking water study, no changes in serum cholesterol or triglycerides were noted in mice exposed to doses up to 3,000 mg/kg/day for 30 days (Wang et al. 2020).

Animal Studies—Elevated Liver Weight and Hypertrophy, Peroxisomal Proliferation, Enzyme Induction. These endpoints are associated with hepatomegaly in animals and may reflect adaptation of the liver to xenobiotic exposure; therefore, they may not be relevant to human health. The European Society of Toxicologic Pathology (ESTP) convened an expert panel to define what constitutes an adverse hepatic effect and whether hepatic effects induced by nuclear receptors such as PPARα, constitutive androstane receptor (CAR), or pregnane X receptor (PXR) are rodent-specific adaptive reactions; the findings of the panel are summarized by Hall et al. (2012). According to these criteria, increased liver weight *without* histological evidence of hepatobiliary damage (degeneration, fibrosis, necrosis, cholestasis) is not considered adverse or relevant for human risk assessment unless at least two of the following three parameters are observed: (1) at least 2–3 times increase in ALT levels; (2) biologically significant change in other biomarkers of hepatobiliary damage (ALP, AST, GGT, etc.); or (3) biologically significant change in another clinical pathology marker indicating liver dysfunction (albumin, bilirubin, bile acids, coagulation factors, cholesterol, triglycerides, etc.). ATSDR has adopted the criteria from Hall et al. (2012) for determining the adversity of the liver effects reported in the rodent following exposure to DEHP since the proposed mechanism of liver toxicity for DEHP is PPAR-mediated (Kushman et al. 2013; Rusyn and Corton 2012); DEHP has also been shown to activate PxR and CAR (Rusyn and Corton 2012) (see ***Mechanisms of Hepatic Toxicity*** at the end of this section). Therefore, these effects are only discussed briefly below, and were not considered adverse effects unless hepatocellular degenerative or necrotic changes or evidence of biliary or other liver cell damage were also present. If parameters other than liver weight, hypertrophy, enzyme induction, and/or peroxisome proliferation were evaluated, the lowest doses associated with the liver weight increases and hepatocellular hypertrophy are noted in the LSE tables even though the dose levels are considered NOAELs. Studies that evaluated parameters associated with hepatomegaly only (and not clinical chemistry and/or histopathology) were not included in Tables 2-1 and 2-2 because they were considered inadequate to assess hepatic toxicity; however, these studies are discussed briefly below.

No evidence of elevated liver weight, hypertrophy, peroxisomal proliferation, or enzyme induction was observed in nonhuman primates following oral exposure to DEHP. No evidence of liver enlargement was observed in monkeys exposed to doses up to 2,500 mg/kg/day for up to 13 weeks (ICI Americas Inc. 1982; Kurata et al. 1998; Rhodes et al. 1986; Satake et al. 2010; Short et al. 1987). Additionally, there was no evidence of peroxisomal proliferation or enzyme induction in monkeys exposed to doses up to 2,500 mg/kg/day for up to 13 weeks (ICI Americas Inc. 1982; Kurata et al. 1998; Rhodes et al. 1986; Short et al. 1987).

In contrast to nonhuman primate findings, oral exposures to DEHP characteristically result in a marked increase in liver weight and hepatocyte hypertrophy in rats and mice. The lowest reported doses associated with these effects in adult, nonpregnant rats and mice were 5 and 180 mg/kg/day, respectively (Sasaki et al. 2003; Zhang et al. 2017). One gestational/lactation exposure study reported increased maternal liver weight in mice at 5 mg/kg/day (Pocar et al. 2012). A large number of additional studies in rats or mice also reported increased liver weight and/or hepatocellular hypertrophy at higher doses (Table 2-2).

In other mammalian species, hypertrophy and/or elevated liver weights have been observed in hamsters exposed to ≥100 mg/kg/day for 14 days (Lake et al. 1984), guinea pigs exposed to 64 mg/kg/day for 1 year (Carpenter et al. 1953), and ferrets exposed to 1,200 mg/kg/day for 14 months (Lake et al. 1976). No evidence of liver enlargement was observed in dogs exposed to 56.6 mg/kg/day for 1 year (Carpenter et al. 1953).

Enlarged livers may be attributable to rapid cell division (hyperplasia), along with cellular hypertrophy, as hepatic hyperplasia appears to be the initial physiological response to DEHP exposure in rats (Busser and Lutz 1987; Smith-Oliver and Butterworth 1987). When rats were exposed to single doses ≥150 mg DEHP/kg, there was an increase in cell division within 24 hours (Berman et al. 1995; Busser and Lutz 1987; Smith-Oliver and Butterworth 1987). During the early stages of a chronic study, repeated oral doses ≥50 mg/kg/day increased mitotic activity when given to rats for 3 consecutive days (Mitchell et al. 1985). The increase in mitosis occurred only in the early stages of treatment and did not persist beyond the first week of exposure in studies with 3–12-month durations (Marsman et al. 1988; Mitchell et al. 1985; Smith-Oliver and Butterworth 1987).

Exposure to DEHP in rats and mice was consistently associated with peroxisomal proliferation. In the only inhalation study that evaluated this endpoint, no exposure-related evidence of peroxisomal proliferation was observed in rats following intermittent exposure to concentrations up to 63 ppm for 4 weeks (Klimisch et al. 1991, 1992). In acute oral rat studies, induction of peroxisomal enzymes and peroxisomal proliferation were observed at doses ≥530 and ≥1,000 mg/kg/day, respectively (Astill et al. 1986; David et al. 1999; Ganning et al. 1989; Hasmall et al. 2000; Lake et al. 1984; Poon et al. 1997; Shin et al. 1999).

Following intermediate-duration oral exposure, evidence of peroxisomal enzyme induction was apparent in rats at doses ≥50 mg/kg/day (Astill et al. 1986; Barber et al. 1987; Cattley et al. 1987; CMA 1986; Exxon Chemical Americas 1990; Ganning et al. 1991; Lake et al. 1984, 1987; Marsman et al. 1988; Mitchell et al. 1985; Rao et al. 1987; Short et al. 1987; Tamura et al. 1990). In mice, peroxisomal enzyme induction was significantly elevated at ≥1,881 mg/kg/day following exposure for 1–13 weeks and ≥292.3 mg/kg/day following exposure for 104 weeks (David et al. 1999); no other studies evaluated peroxisomal enzymes in mice. Observed changes in peroxisomal enzymes included induction of enzymes responsible for fatty acid catabolism (palmitoyl-CoA oxidase, enoyl-CoA hydratase, carnitine acyltransferase, and α-glycerophosphate dehydrogenase) in rats and mice after exposure to DEHP by factors as great as 1,500%. Findings for induction of peroxisomal catalase in rats are mixed, with some dietary studies reporting decreased catalase activity (Ganning et al. 1989; Rao et al. 1987), increased catalase activity (Conway et al. 1989; Ganning et al. 1991; Perera et al. 1986; Tamura et al. 1990), or no change in activity (Elliott and Elcombe 1987; Perera et al. 1986). The findings did not show a clear pattern with respect to strain, sex, or exposure duration, and may be mediated by factors unrelated to DEHP exposure.

Findings for peroxisomal proliferation in other mammalian species are limited. In hamsters, slight peroxisomal proliferation was observed following a 14-day exposure to 1,000 mg/kg/day; however, no changes were observed in peroxisomal enzymes (Lake et al. 1984). Peroxisomal proliferation was not observed in guinea pigs exposed to 950 mg/kg/day for 4 days (Hasmall et al. 2000). Catalase was decreased in ferrets exposed to 1,200 mg/kg/day for 14 months (Lake et al. 1976).

The mixed-function oxidase (MFO) system appears to be affected by DEHP in rodents (Ganning et al. 1991; Short et al. 1987). Significant induction of fatty acid omega hydroxylase and P-450 4A1 messenger ribonucleic acid (mRNA) were reported following DEHP administration to rats (Sharma et al. 1988, 1989). Increases in hepatic levels of cytochrome P-450, NADPH cytochrome c reductase, lauryl-11- and 12-hydroxylase, ethoxycoumarin-O-deethylase, ethylmorphine-N-demethylase, and/or aniline hydroxylase were induced by DEHP exposure of rats to doses ≥50 mg/kg/day (Barber et al. 1987; CMA 1986; Ganning et al. 1991; Lake et al. 1984; Mitchell et al. 1985; Short et al. 1987) and in ferrets exposed to 1,200 mg/kg/day for 14 months (Lake et al. 1976). No exposure-related changes were observed in the MFO system in dogs exposed to 56.6 mg/kg/day for 1 year (Carpenter et al. 1953).

Mechanisms of Hepatic Toxicity. Kushman et al. (2013) identified nine mechanistic events for DEHP and its metabolites in the liver based on a survey of several highly cited and diverse reviews (Caldwell

2012; Guyton et al. 2009; Klaunig et al. 2003; McKee 2000; Melnick 2001; Peters et al. 2005; Roberts et al. 2007; Rusyn and Corton 2012; Rusyn et al. 2006). The key mechanistic events include: (1) PPAR activation (most likely α); (2) peroxisome proliferation; (3) cell proliferation; (4) activation of other nuclear receptors; (5) Kupffer cell activation; (6) suppression of hepatocellular apoptosis; (7) oxidative stress; (8) inhibition of gap-junctional intracellular communication (GJIC); and (9) genotoxicity. The role of specific key events in rodent liver cancer is described in Section 2.19 (Mechanisms of Liver Cancer).

PPAR activation in the liver of mice and rats by DEHP and metabolites is well established (Rusyn and Corton 2012). MEHP activates mouse and human PPARα, PPARδ, and PPARγ. PPARα is expressed at higher levels in mouse and rat liver compared to human liver. In the liver, PPARα plays a role in fatty acid uptake and transport, ketogenesis, and lipogenesis. The hallmarks of PPARα activation include: (1) an increase in the number and size of peroxisomes (i.e., peroxisome proliferation); (2) increased gene expression, protein level, or activity of acyl Co-A oxidase or CYP4A (i.e., ω-lauric acid hydroxylase); and (3) increased levels of carnitine acyl Co-A transferase. These effects are generally observed in rats and mice, but were not seen in studies of nonhuman primates (i.e., marmosets and Cynomolgus monkeys). PPARα is also responsible for the burst of hepatocyte proliferation that is seen with peroxisome proliferating compounds, including DEHP, in rodents (i.e., proliferation is not observed in PPARα-null mice).

Induction of peroxisomal and microsomal enzymes mediated by PPARα contributes to an increase in the formation of ROS (measure of oxidative stress) in the rodent liver. Glutathione peroxidase and superoxide dismutase are important elements in the cellular defenses against free radical oxygen; however, reduction in these enzymes has been reported following acute-, intermediate-, and chronic-duration oral exposure in rats (Conway et al. 1989; Elliott and Elcombe 1987; Perera et al. 1986; Tamura et al. 1990) and chronic-duration oral exposure in ferrets (Lake et al. 1976). Depletion of these enzymes may not be detected due to changes in carbohydrate metabolism, indicating increased hepatic glucose utilization (Gerbracht et al. 1990; Lake et al. 1976; Mitchell et al. 1985). These metabolic findings support increased demand for hepatic glucose utilization, which would produce the reducing equivalents necessary for the activity of glutathione peroxidase. Additional evidence of oxidative stress includes increased levels of lipid ubiquinone (Turunen and Dallner 1998) and cellular ubiquinone (Nair and Kurup 1987b) in rats following intermediate-duration oral exposure to DEHP.

DEHP and its metabolites have been shown to activate other nuclear receptors in human cells including the estrogen receptor, human pregnane X-receptor and the constitutive androstane receptor (CAR);

however, the role of activation of these receptors in liver toxicity has not been fully elucidated (Rusyn and Corton 2012). Activation of Kupffer cells in the rat liver following exposure to DEHP resulted in the production of ROS as measured by spin trapping and electron spin resonance techniques. Kupffer cell activation may also result in release of inflammatory cytokines and mitogenic growth factors in the liver (Roberts et al. 2007; Rusyn and Corton 2012), and suppression of apoptosis and increased deoxyribonucleic acid (DNA) synthesis were also observed in the liver of rats and mice exposed to DEHP and MEHP (Rusyn and Corton 2012).

The effect of DEHP on liver metabolism might be mediated by changes in the structure of the cell membranes. Both membrane proteins and lipids are altered with DEHP exposure (Bartles et al. 1990; Edlund et al. 1987; Ganning et al. 1987; Gupta et al. 1988). Following 15 days of dietary exposure to 1,000 mg/kg/day DEHP, the concentration of membrane protein CE-9 was increased in rats. This protein appears to be related to transport of the biochemical signal that stimulates peroxisome proliferation. Other membrane protein concentrations were decreased with DEHP exposure in rats, including epidermal growth factor receptor, asialoglycoprotein receptor, dipeptidylpeptidase-IV, HA-312, and HA-4 (Bartles et al. 1990; Gupta et al. 1988). There were increases in the concentrations of the membrane lipids, dolichol and dolichol phosphate, upon the introduction of DEHP into the diet of rats (Edlund et al. 1987; Ganning et al. 1987). Dolichol phosphate participates in the synthesis of membrane glycoproteins. Accordingly, glycoprotein membrane receptor sites could be affected by DEHP through this mechanism, leading to altered movement of materials across membranes and signaling changes in cell metabolism.

Hepatic damage may also be mitigated in part due to the reaction of hydrogen peroxide with cellular lipids. Slight, but significant, increases in malondialdehyde and conjugated dienes (markers for the reaction of peroxides with fatty acids) were seen in rat hepatic cells following 28 days of exposure to 2,000 mg/kg/day DEHP (Elliott and Elcombe 1987). In a separate study, there was no increase in oxidized lipids, as indicated by malondialdehyde concentrations, in exposed rat livers following 79 weeks of dietary exposure to 1,500 mg/kg/day DEHP (Tamura et al. 1990). Lipofuscin deposits, a long-term marker for lipid reactions with peroxides, were identified in the livers of rats exposed to between 500 and 2,000 mg/kg/day DEHP for their lifetime (Price et al. 1987). Inhibition of GJIC in rodent liver was also correlated with PPARα-mediated peroxisome proliferation (McKee 2000).

Summary. Human data on hepatic effects of DEHP are limited but suggest that occupational exposure levels may be associated with increased serum liver enzyme levels and decreased plasma cholinesterase activity. In studies of general population exposures, urinary metabolite levels were generally not

consistently associated with changes in triglyceride or cholesterol levels; there were no studies of other hepatic endpoints in humans exposed to DEHP in the environment or in consumer products. In rodents, high DEHP doses resulted in degenerative and necrotic hepatic changes. Dogs and monkeys are less likely to experience changes in the liver after exposure. At lower exposure levels, the predominant noncancer effects observed in laboratory animals exposed to DEHP included elevated liver weight, hypertrophy, peroxisome proliferation, and/or enzyme induction. As discussed above, the adversity and human relevance of these findings are unclear.

2.10 RENAL

Overview. A limited number of epidemiological studies evaluated renal clinical chemistry and/or urinalysis parameters in DEHP-exposed populations. Data in animals following inhalation exposure are limited, but several oral animal studies evaluated kidney function, weight, and histology.

Epidemiology Studies. In a study of 352 Chinese workers exposed to DEHP at three different PVC manufacturing facilities (average exposures ranging between 233 and 707 $\mu g/m^3$ DEHP in the three factories), serum urea and creatinine levels did not differ from those in 104 unexposed workers (Wang et al. 2015).

There is some evidence of altered renal clinical chemistry in Taiwanese children exposed to foods contaminated with DEHP (duration of exposure time unknown). Tsai et al. (2016) reported higher urinary albumin/creatinine ratio (ACR) in a group of exposed Taiwanese children, compared to an unexposed group. ACR indicates elevated protein levels in the urine and is a biomarker for kidney disease. Exposed children also had a higher prevalence of microalbuminuria associated with the highest intake of contaminated foods (estimated to be >0.05 mg/kg/day), compared with unexposed children (Tsai et al. 2016; Wu et al. 2018). However, serum blood urea nitrogen (BUN) and creatinine levels in exposed children did not differ from those in unexposed children, and there were no differences in urinalysis findings (protein, occult blood, or erythrocyte or leukocyte counts) (Wu et al. 2013).

A cross-sectional study (Trasande et al. 2014) using 2009–2010 NHANES data on 667 children also reported an association between higher levels of DEHP metabolites in urine and increasing urinary ACR. However, the odds of micro- or macroalbuminuria (ACR ≥30 mg/g) were not increased in children with higher levels of DEHP metabolites in urine (Trasande et al. 2014). In contrast, a cross-sectional study in Taiwanese children and adolescents (7–<18 years old; not among the children exposed to contaminated

food) did not find an association between urinary levels of DEHP metabolites and ACR, BUN, or odds of microalbuminuria (Chang et al. 2020). In Taiwanese adults, increased urinary DEHP metabolites were associated with increased serum BUN (Chang et al. 2020). Across the entire population, no association was observed between ACR or microalbumin and DEHP exposure. However, odds of microalbuminuria (microalbumin >1.9 mg/dL) were increased in individuals in the highest tertile of estimated DEHP intake (≥0.003 mg/kg/day), compared to the lowest (<0.001 mg/kg/day).

Animal Studies. Following inhalation exposure to DEHP, no changes in renal serum chemistry, kidney weight, or kidney histology were observed in rats exposed nose-only to concentrations up to 63 ppm for 4 weeks (5 days/week, 6 hours/day) (Klimisch et al. 1992).

In orally exposed nonhuman primates, no changes in clinical chemistry measures of renal function, urinalysis parameters, or kidney weight or histology were observed in marmoset monkeys exposed to 2,000 mg/kg/day for 14 days (ICI Americas Inc. 1982; Rhodes et al. 1986). Similarly, no exposure-related changes were observed in clinical chemistry or kidney weight or histology in monkeys exposed to doses up to 2,500 mg/kg/day for 13 weeks (Kurata et al. 1998; Satake et al. 2010).

Histopathological changes in the kidney have been observed in multigeneration and chronic-duration oral studies in rats. In a 3-generation study in Sprague-Dawley rats, increased incidences of kidney lesions (medullary mineralization and tubular dilation) were observed in F1 and F2 parental males and F2 parental females at doses ≥447 mg/kg/day, but not ≤57 mg/kg/day (Blystone et al. 2010; NTP 2005). Similarly, in 2-generation studies in Wistar rats, renal tubule dilation and renal pelvis calcification were observed in F1 adults at 1,088 mg/kg/day, but not ≤1,040 mg/kg/day (Schilling et al. 1999, 2001). Consistent with the observation that renal effects occur at higher doses, no kidney lesions were observed in a combination chronic/2-generation study in Sherman rats exposed to doses up to 200 mg/kg/day (Carpenter et al. 1953). At chronic-duration dietary exposures ≥789 mg/kg/day, increased severity of chronic progressive nephropathy was observed in both sexes (David et al. 2000a); no exposure-related changes in kidney histology were observed at doses ≤774 mg/kg/day (David et al. 2000a; Kluwe et al. 1982a; NTP 1982). Additionally, Rao et al. (1990) did not observe histopathological changes in the rat kidney following exposure to 1,900 mg/kg/day for 108 weeks.

In shorter-duration studies, no histopathological changes were observed in most rat studies at doses up to 10,000 mg/kg/day for up to 4 weeks (Astill et al. 1986; Barber et al. 1987; CMA 1986; Dalgaard et al. 2000; NTP 1982), up to 3,000 mg/kg/day for up to 13 weeks (Dalgaard et al. 2000; Myers 1992b; NTP

1982; Poon et al. 1997; Shaffer et al. 1945), or up to 1,440 mg/kg/day for 17 weeks (Gray et al. 1977). However, a 4-week study in male rats reported increased glomerular degeneration, congestion, and mononuclear cell infiltration at gavage doses ≥100 mg/kg/day (Aydemir et al. 2018).

Histopathological changes in the kidney have also been reported in intermediate- and chronic-duration studies in mice. Acute renal inflammation, characterized by tubular necrosis, tubular dilation, tubular regeneration, and occasional neutrophilic infiltrates, was observed in male and female mice after exposure to dietary doses of 6,922 and ≥2,888 mg/kg/day, respectively, for 28 days (Myers 1992a). These lesions were not observed in male or female mice exposed to oral doses up to 2,600 mg/kg/day for 4–13 weeks (Myers 1992a; NTP 1982; Xu et al. 2019). Tubular regeneration was also observed in male and female mice exposed to 1,100 mg/kg/day (only dose tested) for 28 weeks; hydronephrosis was also observed in exposed females (Toyosawa et al. 2001). One intermediate-duration study in mice reported glomerular damage (consistent with hypertensive renal injury) and increased inflammatory cell infiltration at doses ≥1 mg/kg/day (Xie et al. 2019). In chronic studies, doses ≥9.5 mg/kg/day resulted in mild glomerulonephritis and cell proliferation in the kidneys of male SV/129 mice (Kamijo et al. 2007). In B6C3F1 mice, chronic progressive nephropathy was observed in both sexes following exposure to doses ≥292.2 mg/kg/day for 104 weeks (David et al. 2000b). However, another 2-year study in B6C3F1 mice only observed an increased incidence of chronic inflammation of the kidney in males at 1,325 mg/kg/day, with incidences comparable to controls at 672 mg/kg/day in males and at doses up to 1,821 mg/kg/day in females (Kluwe et al. 1982a; NTP 1982).

There is limited evidence for impaired renal function in intermediate-duration studies. Following dietary exposure for 13 weeks, serum BUN levels were slightly, but significantly, elevated by 24–47% in male and female F344 rats at ≥261.2 and ≥850.1 mg/kg/day, respectively (Myers 1992b). In a 4-week gavage study, serum urea was increased by approximately 50% at ≥200 mg/kg/day in male Wistar rats (Aydemir et al. 2018). Additionally, in a 17-week dietary study, both renal concentrating and diluting ability were reduced at week 17 in female rats exposed to 1,414 mg/kg/day, suggesting mild renal functional impairment (23% increase in urine volume in the concentrations test; 47% decrease in urine volume in the dilution test) (Gray et al. 1977). However, no changes in urinalysis and/or clinical chemistry parameters were observed in rats exposed to doses up to 1,440 mg/kg/day for 13–17 weeks (Gray et al. 1977; Kim et al. 2018c; Poon et al. 1997) or doses up to 939 mg/kg/day for 2 years (David et al. 2000a). In a chronic study in SV/129 mice, doses ≥9.5 mg/kg/day resulted in increased protein in the urine (Kamijo et al. 2007); however, no changes in urinalysis parameters were observed in B6C3F1 mice exposed to doses up to 1,458 mg/kg/day for 2 years (David et al. 2000b). An intermediate-duration study in C57Bl/6 mice

reported a 43% increase in serum creatinine following exposure to 300 mg/kg/day for 35 days (Li et al. 2018). In other mouse studies, no exposure-related changes were observed in clinical chemistry measures following intermediate-duration (28 days) exposure to doses up to 7,899 mg/kg/day (Myers 1992a) or chronic-duration (2 years) exposure to doses up to 1,458 mg/kg/day (David et al. 2000b; Kamijo et al. 2007).

Absolute and/or relative kidney weight increases of >10% were observed in several intermediate- or chronic-duration rat studies at doses ≥100 mg/kg/day (Table 2-2) and in acute-duration studies following exposure to 1,000 mg/kg/day (Hellwig et al. 1997). In a Hershberger assay, absolute kidney weights were increased >10% at ≥40 mg/kg/day in castrated rats; no changes in kidney weights were observed in castrated rats supplemented with testosterone propionate at doses up to 400 mg/kg/day (Kim et al. 2018b). In other studies, kidney weight changes did not occur in other rat studies at acute-duration doses of 500–2,100 mg/kg/day (Astill et al. 1986; Dostal et al. 1987; Lee and Koo 2007) or intermediate-duration doses up to 2,101 mg/kg/day (Barber et al. 1987; Grande et al. 2006; Schilling et al. 1999). In mouse studies, relative kidney weight was increased in female mice exposed to 1,100 mg/kg/day for 26 weeks (Toyosawa et al. 2001) and absolute kidney weight (without a significant change in body weight) was increased in male mice exposed to 400 mg/kg/day for 28 days (Xu et al. 2019). However, no kidney weight changes occurred in other mouse studies following exposure to intermediate-duration doses up to 7,899 mg/kg/day or chronic doses up to 48.5 mg/kg/day (Kamijo et al. 2007; Myers 1992a). In studies reporting kidney weight changes, decreased body weights were often observed, and only rarely were renal weight changes associated with histopathological changes (Blystone et al. 2010; NTP 2005; Schilling et al. 2001; Toyosawa et al. 2001) or impaired function (Gray et al. 1977; Myers 1992b).

The relevance of the kidney effects observed in the dietary studies in rats and mice is unclear. Some of the findings (David et al. 2000a, 2000b) suggest exacerbation of typically observed age-, species-, and/or sex-related lesions following DEHP exposure in the absence of impaired kidney function. However, impaired kidney function and kidney lesions were also reported in young rats following developmental exposure to doses ≥0.25 mg/kg/day in some studies (Arcadi et al. 1998; Wei et al. 2012), indicating that the developing kidney may be sensitive to DEHP exposure; see Section 2.17 (Developmental) for more details. Unlike hepatic findings, renal lesions observed in mice do not appear to be primarily associated with PPARα activation, because both wild-type and PPARα knockout (-/-) mice develop kidney lesions after intermediate-duration dietary exposure (Kamijo et al. 2007; Ward et al. 1988). In fact, Kamijo et al. (2007) proposed that PPARα activation protects against DEHP-induced renal toxicity because PPARα knockout (-/-) mice showed increased sensitivity to renal toxicity compared with wild-type mice

following chronic-duration dietary exposure to DEHP, including increased urinary protein, serum BUN and creatinine, and indices of glomerular lesions (cell proliferation and mesangial expansion indices). Mitochondrial dysfunction and oxidative stress were suggested as possible mechanisms for DEHP-induced nephrotoxicity based on experiments in cultured embryonic human kidney cells (HEK-293 cells) (Ashari et al. 2020).

In other mammalian species, no adverse renal effects were seen in guinea pigs or dogs exposed to doses up to 64 or 56.6 mg/kg/day, respectively, for 1 year (Carpenter et al. 1953).

Summary. Human data regarding renal effects following DEHP exposure are extremely limited and inconsistent. There is some evidence that the kidney is a sensitive target of DEHP toxicity in animals following oral exposure. However, most of the available studies observed kidney damage in animals only at high doses.

2.11 DERMAL

Human Studies. No studies of dermal effects in humans exposed to DEHP by inhalation or oral exposure were located. In an early patch test study, no evidence of dermal irritation or skin sensitization was reported after undiluted DEHP (dose not specified) was applied to 23 volunteers on the skin of the back and under occluded conditions for 7 days, followed by a challenge application 10 days later (Shaffer et al. 1945).

Animal Studies. No studies were located regarding dermal effects in animals following inhalation exposure to DEHP.

No histopathological changes in the skin were observed following intermediate-duration oral exposure to DEHP in marmoset monkeys exposed to doses up to 2,500 mg/kg/day (Kurata et al. 1998), rats exposed to doses up to 419.3 mg/kg/day (Poon et al. 1997), or mice exposed to 1,100 mg/kg/day (Toyosawa et al. 2001). In 2-year dietary studies, no histopathological skin lesions were observed in rats or mice at DEHP doses up to 774 or 1,821 mg/kg/day, respectively (Kluwe et al. 1982a; NTP 1982).

A single dose of up to 20 mL/kg (19,700 mg/kg) DEHP was applied to rabbit skin for 24 hours using a modified FDA cuff test procedure. Despite this dose resulting in the death of 2/6 rabbits, there was no evidence of dermal injury caused by DEHP during the 14-day observation period (Shaffer et al. 1945).

2.12 OCULAR

Human Studies. No studies were located regarding ocular effects in humans after exposure to DEHP.

Animal Studies. No studies were located regarding ocular effects in animals following inhalation exposure to DEHP.

No ocular effects were noted during an ophthalmological examination of rats following a 13-week exposure to DEHP in the diet at doses up to 1,857.6 mg/kg/day (Myers 1992b). No other studies performed ophthalmological examination following oral DEHP exposure.

In other studies, no histopathological changes in the eyes were observed in marmoset monkeys exposed to doses up to 2,500 mg/kg/day for 13 weeks (Kurata et al. 1998), rats exposed to doses up to 419.3 mg/kg/day (Poon et al. 1997), or mice exposed to 1,100 mg/kg/day for 26 weeks (Toyosawa et al. 2001).

There was no necrosis of rabbit cornea after ocular exposure to a single dose of 0.5 mL (495 mg) DEHP, but a slight transient congestion of the eyelids was observed (Shaffer et al. 1945). These data indicate that neat DEHP has a low potential for ocular irritation in rabbits.

2.13 ENDOCRINE

Overview. Various endocrine organs have been evaluated after exposure to DEHP. This section focuses on the pancreas, adrenal gland, pituitary gland, and thyroid/parathyroid glands. While reproductive organs also have endocrine function, these organs (testes, ovaries) and the hormones that they produce are discussed in Section 2.16 (Reproductive). Human epidemiological data have evaluated potential associations between DEHP exposure and thyroid hormone levels and cord blood glucocorticoids. Data regarding potential endocrine effects in animals following DEHP exposure were available from one inhalation study and numerous oral studies.

Epidemiology Studies—Thyroid Function. Effects of DEHP exposure on thyroid function (serum levels of triiodothyronine [T3], thyroxine [T4], and thyroid stimulating hormone [TSH]) have been evaluated in 19 epidemiological studies in which DEHP exposure was evaluated using urinary metabolite biomarkers (Table 2-6).

Table 2-6. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Pregnant women				
Huang et al. 2018 Cohort/cross-sectional, 98 pregnant women referred for amniocentesis (mean age 35 years), Taiwan, China	TSH	ΣDEHP	GW 18: GM (95% CI): 21.64 (16.44, 28.25) ng/mL GW 26: 30.68 (24.51, 38.39) GW 39: 39.34 (31.60, 48.97)	↔
		MEHP	GW 18: 2.43 (1.67, 3.52) GW 26: 3.45 (2.43, 4.91) GW 39: 2.49 (1.60, 3.87)	↔
		MEHHP	GW 18: 2.67 (1.75, 4.08) GW 26: 5.33 (3.63, 7.82) GW 39: 9.69 (7.27, 12.91)	↔
		MEOHP	GW 18: 3.41 (2.45, 4.75) GW 26: 5.36 (4.06, 7.08) GW 39: 8.38 (6.68, 10.52)	↓
		MECPP	GW 18: 6.15 (4.37, 8.65) GW 26: 9.89 (7.95, 12.30) GW 39: 12.46 (10.03, 15.50)	↔
	TT3	ΣDEHP, MEHP, MEHHP, or MEOHP	See above	↔
		MECPP	See above	↓
	TT4 and FT4	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	See above	↔
		No significant association was seen in analyses of maternal serum hormone levels stratified by visit, or in analyses of the relationship between maternal urinary metabolite levels and cord serum hormone levels.		

Table 2-6. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Huang et al. 2007 Cross-sectional, 76 pregnant women referred for amniocentesis (mean age 22.6 years), Taiwan, China	FT4 or TT4	MEHP	IQR: 31.4–121.0 µg/g Cr	↔
Johns et al. 2016 Case-control, 439 pregnant women (116 cases of preterm birth and 323 term birth controls), United States (Massachusetts)	**TSH**	ΣDEHP	GM (GSD): Median GW 9.71: 0.39 (3.16) µmol/L (SG-adj) Median GW 17.9: 0.38 (3.01) Median GW 26: 0.32 (3.04) Median GW 35.1: 0.42 (3.18)	↔
		MEHP	GM(GSD): Median GW 9.71: 10.6 (3.52) µg/L (SG-adj) Median GW 17.9: 10.9 (3.39 Median GW 26: 9.46 (3.28) Median GW 35.1: 9.83 (3.52)	↓
		MEHHP	Median GW 9.71: 34.7 (3.37) Median GW 17.9: 34.8 (3.10) Median GW 26: 27.2 (3.21) Median GW 35.1: 9.83 (3.33)	↔
		MEOHP	Median GW 9.71: 18.6 (3.28) Median GW 17.9: 18.3 (3.03) Median GW 26: 15.6 (3.19) Median GW 35.1: 20.9 (3.22)	↔
		MECPP	Median GW 9.71: 44.4 (3.35) Median GW 17.9: 42.6 (3.25) Median GW 26: 36.8 (3.31) Median GW 35.1: 49.3 (3.35)	↔
	TT3 and FT4	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	See above	↔

Table 2-6. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
	TT4	ΣDEHP, MEHHP, MEOHP, or MECPP	See above	↔
		MEHP	See above	↑
		Repeated measures analysis with cases and controls combined.		
Johns et al. 2015 Cohort/cross-sectional, 106 pregnant women (age 18–40 years), Puerto Rico	**FT4**	**ΣDEHP**	NR	↓
		MEHP	GWs 16–20: IQR: 1.61–6.36 ng/mL (SG-adj) GWs 24–28: 1.69–6.73	NR
		MEHHP	GWs 16–20: 6.14–19.9 GWs 24–28: 7.28–16.9	NR
		MEOHP	GWs 16–20: 5.57–16.5 GWs 24–28: 6.22–14.8	NR
		MECPP	GWs 16–20: 12.7–31.4 GWs 24–28: 13.4–29.3	NR
	TSH and FT3	ΣDEHP	NR	↔
		Cross-sectional analysis (same day serum and urine samples) using visit 3 (GWs 24–28) data only; no significant association seen with visit 1 (GWs 16–20) data only or in longitudinal analysis.		
Kuo et al. 2015 Cohort, 148 mother-child pairs, Taiwan	TSH (cord blood)	MEHP	IQR: 8.19–19.34 µg/g Cr	↔
		MEHHP	14.84–33.81	↔
		MEOHP	14.68–31.59	↔
Villanger et al. 2020a, 2020b Cohort/cross-sectional with nested case-control, 1,072 pregnant women (534 cases of mothers of children with diagnosed ADHD, 538 random controls; mean age at delivery 30.24 years), Norway	TSH, FT3, TT3, FT4, TT4	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP, MCMHP)	GW 17: 20th–80th percentile: 0.17–0.41 µmol/L	↔

Table 2-6. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Yao et al. 2016 Cohort/Cross-sectional, 2,521 pregnant women (mean age 25 years), China	**TSH**	**MEHP or MEHHP**	NR	↑
		MEOHP	NR	↔
	TT3, TT4, or FT4	**MEHP or MEHHP**	NR	↓
		MEOHP	NR	↔
	No associations were observed between maternal DEHP metabolite levels and cord serum thyroid hormone levels.			
Other populations				
Al-Saleh et al. 2019a Cross-sectional, 599 male partners (mean age 37.86 years) of infertile couples, Saudi Arabia	**TSH**	ΣDEHP	IQR: 0.161–0.433 µmol/L	↔
		MEHP	IQR: 9.467–22.368 µg/L	↔
		MEHHP	5.889–20.496	↔
		MEOHP	9.875–28.432	↔
		MECPP	17.044–53.328	↔
Boas et al. 2010 Cross-sectional, 758 children (age 4–9 years), Denmark	TSH, TT3, TT4, FT3, or FT4	ΣDEHP	NR	↔
		MEHP	Males: IQR: 4.1–11 µg/g Cr Females: 4.1–12	↔
		MEHHP	Males: 33–84 Females: 36–81	↔
		MEOHP	Males: 17–42 Females: 18–41	↔
		MECPP	Males: 29–68 Females: 33–75	↔
	Cr-corrected analysis for all children (girls and boys combined); p>0.05 for all.			
Dirtu et al. 2013 Case-control, 152 obese individuals and 43 non-obese controls (age 19–59 years), Belgium	**TSH**	**ΣDEHP**	**Controls**: IQR: 27–53 ng/mL	↑
			Cases: 30–61	↔
		MEHP	Controls: 2–5	↔
			Cases: 2–5	↔
		MEHHP	Controls: 9–19	↔
			Cases: 10–25	↔

Table 2-6. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
		MEOHP	**Controls:** 3–9	↑
			Cases: 4–11	↔
		MECPP	**Controls:** 12–20	↑
			Cases: 12–22	↔
	FT4	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	Controls: see above	↔
			Cases: see above	↔
		Gender-specific results also did not show any significant associations for DEHP metabolites.		
Huang et al. 2017 Cross-sectional, 279 adults (age ≥18 years, mean age 53.4 years) and 79 minors (age <18 years, mean age 12.6 years), Taiwan	**FT4 (adults)**	ΣDEHP	IQR: 0.16–0.36 µmol/g Cr	↔
		MEHP	IQR: 3.25–15.08 µg/g Cr	↓
		MEHHP	13.36–30.51	↔
		MEOHP	8.22–20.02	↓
		MECPP	16.43–38.73	↔
		MCMHP	0.33–7.16	↔
	TT4 (adults)	**ΣDEHP, MEHHP**	See above	↓
		MEHP, MEOHP, MECPP, or MCMHP	See above	↔
	TSH, TT3 (adults)	ΣDEHP, MEHP, MEHHP, MEOHP, MECPP, or MCMHP	See above	↔
	TT3 (minors)	ΣDEHP	IQR: 0.17–0.53 µmol/g Cr	↔
		MEHP	IQR: 2.38–12.17 µg/g Cr	↑
		MEHHP	14.45–44.11	↔
		MEOHP	9.42–27.98	↔
		MECPP	18.88–55.05	↔
		MCMHP	1.71–10.99	↔

Table 2-6. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
	TSH, FT4, TT4 (minors)	ΣDEHP, MEHP, MEHHP, MEOHP, MECPP, or MCMHP	See above	↔
Huang et al. 2020a Cross-sectional, 266 adults (age ≥18 years, mean age 53.6 years), Taiwan	**TT4**	**ΣDEHP**	GM (95% CI): 0.199 (0.180, 0.219) nmol/mL	↓
		MEHP	GM (95% CI): 3.689 (2.955, 4.604) ng/mL	↔
		MEHHP	15.90 (14.01, 18.04)	↓
		MEOHP	8.346 (7.128, 9.772)	↓
		MECPP	17.57 (15.11, 20.44)	↓
		MCMHP	1.524 (1.219, 1.906)	NR
	TSH, TT3, or FT4	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	See above	↔
Huang et al. 2020b Cohort, 166 children and adolescents examined at 3 clinical visits over 4 years post-exposure to phthalate-tainted food (age 2–18 years, mean age at visit 1: 6.1 years, visit 2: 7.9 years, and visit 3: 9.8 years), Taiwan	**FT4**	**ΣDEHP**	Visit 1: IQR: 0.08–0.26 nmol/L Visit 2: 0.08–0.32 Visit 3: 0.06–0.23	↑
		MEHP	Visit 1: IQR: 1.77–9.98 ng/mL Visit 2: 3.83–13.7 Visit 3: 2.57–8.63	↔
		MEHHP	Visit 1: 11.4–38.84 Visit 2: 12.1–45.4 Visit 3: 7.79–35.5	↑
		MEOHP	Visit 1: 7.54–29.42 Visit 2: 7.66–29.4 Visit 3: 6.01–21.6	↑
	TSH, TT3, or TT4	ΣDEHP, MEHP, MEHHP, or MEOHP	See above	↔

Table 2-6. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Kim et al. 2017a, 2017b Cross-sectional, 1,829 adolescents and adults (age ≥12 years), United States (NHANES)	**TSH**	MEHP	IQR: 0.780–5.30 ng/mL	↔
		MEHHP	9.10–46.6	↑
		MEOHP	5.20–25.6	↑
		MECPP	14.7–65.9	↑
	FT3, TT3	MEHP, MEHHP, MEOHP, MECPP	See above	↔
	FT4	MEHP or MECPP	See above	↔
		MEHHP or MEOHP	See above	↓
	TT4	MEHP	See above	↔
		MEHHP, MEOHP, or MECPP	See above	↓
Meeker and Ferguson 2011 Cross-sectional, 1,346 adults (age ≥20 years) and 329 adolescents (age 12–19 years), United States (NHANES)	**TSH**	MEHP	Adolescent: IQR: <LOD–4.5 µg/g Cr:	↔
			Adult: <LOD–5.20	↔
		MEHHP	Adolescent: 10.3–45.32	↔
			Adult: 9.84–37.0	↑
		MEOHP	Adolescent: 5.79–24.74	↔
			Adult: 5.43–20.5	↑
		MECPP	Adolescent: 16.7–64.8	↔
			Adult: 15.4–50.8	↑
	TT3	**MEHP or MECPP**	**Adolescent:** see above	↑
			Adult: see above	↔
		MEHHP	**Adolescent:** see above	↑
			Adult: see above	↓
		MEOHP	**Adolescent:** see above	↑
			Adult: see above	↓
	TT4	**MEHP, MEHHP, MEOHP, or MECPP**	Adolescent: see above	↔
			Adult: see above	↓

Table 2-6. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
	FT3 and FT4	MEHP, MEHHP, MEOHP, or MECPP	Adolescent: see above	↔
			Adult: see above	↔
Meeker et al. 2007 Cross-sectional, 408 male partners of sub-fertile couples (age 18–55 years), United States (Massachusetts)	**TT3**	**MEHP**	IQR: 3.16–21.3 ng/mL (SG-adj)	↓
		MEHHP	23.4–113	↔
		MEOHP	16.3–71.3	↔
	TSH and FT4	MEHP, MEHHP, MEOHP	See above	↔
Morgenstern et al. 2017 Cohort/Cross-sectional, 181 mother-child pairs (99 girls, 82 boys; age 3 years) and 229 children (120 girls, 109 boys; age 3 years), United States (New York City)	**FT4 (children)**	**MEHP**	**Maternal:** 5.7 (4.7, 6.9)	↑
			Child: 3.2 (2.8, 3.7)	↔
		MEHHP	Maternal: 23.6 (19.6, 28.5)	↔
			Child: 32.8 (27.9, 38.5)	All: ↔ Boys: ↔ **Girls: ↓**
		MEOHP	Maternal: 19.7 (16.4, 23.7)	↔
			Child: 19.2 (16.4, 22.5)	All: ↔ Boys: ↔ **Girls: ↓**
		MECPP	Maternal: 41.6 (35.2, 49.2)	↔
			Child: 61.0 (52.9, 70.3)	↔
	TSH (children)	MEHP, MEHHP, MEOHP, MECPP	Maternal: see above	↔
			Child: see above	↔
Park et al. 2017 Cross-sectional, 6,003 adults (2,638 men, 3,365 women; age ≥20 years), Korea	**TSH**	ΣDEHP	Total: IQR: 31.54–89.41 µg/L	↔
			Men: 34.17–90.02	↔
			Women: 29.64–88.74	↔
		MEHHP	Total: 10.72–32.14	↔
			Men: 11.63–32.73	↔
			Women: 9.850–31.71	↔

Table 2-6. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
		MEOHP	**Total:** 7.670–22.35	↑
			Men: 8.050–22.16	↔
			Women: 7.289–22.58	↔
		MECPP	Total: 12.84–34.77	↔
			Men: 13.53–34.63	↔
			Women: 11.97–35.40	↔
	TT3	ΣDEHP, MEHHP, MEOHP, or MECPP	Total: see above	↔
			Men: see above	↔
			Women: see above	↔
	TT4	**ΣDEHP or MEHHP**	**Total:** see above	↓
			Men: see above	↓
			Women: see above	↔
		MEOHP or MECPP	Total: see above	↔
			Men: see above	↔
			Women: see above	↔
Souter et al. 2020a, 2020b Cross-sectional, 558 sub-fertile women seeking fertility treatment (median age 34.0 years), United States (Massachusetts)	**FT3**	**ΣDEHP**	NR	↓
		MEHP	IQR: <LOD–4.30 µg/L	↔
		MEHHP	3.50–23.6	↔
		MEOHP	2.30–15.2	↔
		MECPP	6.30–38.2	↑
	TT3, FT4	**ΣDEHP**	NR	↓
		MEHP, MEHHP, MEOHP, or MECPP	See above	↔
	TT4	**ΣDEHP**	NR	↓
		MEHHP	See above	↓
		MEHP, MEOHP, or MECPP	See above	↔

Table 2-6. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
	TSH	ΣDEHP	NR	↔
		MEHP, MEHHP, MEOHP, or MECPP	See above	NR
Weng et al. 2017 Cohort/Cross-sectional, 189 children (92 boys, 97 girls; age 9–10 years), Taiwan	**FT3**	**ΣDEHP**	33rd–66th percentile (adjusted for Cr): 25.34–49.92 µg/L	↑ **(all, boys)** ↔ girls
		MEHP	2.74–6.42	↔
		MEHHP	13.04–25.93	↑ **(all, boys)** ↔ (girls)
		MEOHP	9.15–17.95	↔ (all, girls) ↑ **(boys)**
	FT4	ΣDEHP, MEHP, or MEHHP	See above	↔
		MEOHP	See above	↔ (all, boys) ↑ **(girls)**

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; ADHD = attention-deficit/hyperactivity disorder; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; FT3 = free triiodothyronine; FT4 = free thyroxine; GM = geometric mean; GSD = geometric standard deviation; GW = gestation week; IQR = interquartile range; LOD = limit of detection; MCMHP = mono-[(2-carboxymethyl)-hexyl] phthalate; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NHANES = National Health and Nutrition Examination Survey; NR = not reported; SG-adj = specific gravity adjusted; TSH = thyroid stimulating hormone; TT3 = total triiodothyronine; TT4 = total thyroxine

Six studies examining thyroid hormone levels in pregnant women did not provided consistent findings. In the largest of these (n=2,521 women; Yao et al. 2016), increased MEHP and MEHHP levels in first trimester urine were associated with decreased free and total T4 and increased TSH levels in maternal serum; no association was observed between total T3 levels and MEHP or MEHPP levels, and MEOHP levels were not associated with any thyroid hormone levels. However, in another large study (n=1,072 women) (Villanger et al. 2020a), the sum of DEHP metabolite levels were not associated with TSH or free or total T3 or T4 in maternal serum during gestation week 17; potential associations with individual metabolites were not evaluated. In a smaller study of 439 pregnant women, increased MEHP levels in maternal urine were associated with increased total T4 and decreased TSH levels in maternal serum during gestation weeks 26 and 35, but not at early gestational time points (Johns et al. 2016). In a small study of 106 pregnant women in Puerto Rico, increased DEHP metabolite levels in urine collected between 24 and 28 weeks of gestation were associated with lower free T4, while there was no association when urine samples collected during weeks 16–20 of gestation were analyzed, or in a longitudinal analysis of the data (Johns et al. 2015). In contrast, no association between MEHP levels in urine collected during gestation week 28 and free or total T4 was observed in a small study of 76 Taiwanese women undergoing amniocentesis (Huang et al. 2007). In a follow-up study of a different group of 98 Taiwanese women undergoing amniocentesis, increased MEOHP levels in the urine were associated with decreased TSH levels and increased MECPP levels were associated with decreased total T3 levels when data were combined across three time-points (one per trimester); none of the metabolites were associated with free or total T4 levels (Huang et al. 2018). In pregnancy cohorts, no associations were observed between maternal urinary DEHP metabolite levels and cord serum thyroid hormone levels (Huang et al. 2018; Kuo et al. 2015; Yao et al. 2016). However, Morgenstern et al. (2017) reported an association between maternal third trimester urinary MEHP levels and increased free T4 serum levels in 3-year-old children; no association was observed with TSH levels in children. Other studies in pregnant women did not evaluate cord or child serum thyroid levels.

Findings in cross-sectional studies of other populations were also inconsistent. Some studies reported associations between DEHP urinary metabolite levels and increased serum TSH in adults and adolescents (Dirtu et al. 2013; Kim et al. 2017a; Meeker and Ferguson 2011; Park et al. 2017), while others did not observed this association in adults (Al-Saleh et al. 2019a; Huang et al. 2017, 2020a; Meeker et al. 2007; Souter et al. 2020a), children or adolescents (Boas et al. 2010; Huang et al. 2017, 2020b; Meeker and Ferguson 2011; Morgenstern et al. 2017), or obese individuals (Dirtu et al. 2013). Similarly, DEHP urinary metabolite levels were associated with decreased free or total T3 in adults in some studies (Meeker et al. 2007; Souter et al. 2020a, 2020b), but increased total or free T3 in children or adolescents

in other studies (Huang et al. 2017; Meeker and Ferguson 2011; Weng et al. 2017). No changes in free or total T3 were associated with DEHP exposure in other studies in adults, adolescents, and children (Boas et al. 2010; Huang et al. 2017, 2020a, 2020b; Kim et al. 2017a, 2017b; Park et al. 2017). DEHP urinary metabolite levels were associated with decreased free and/or total T4 in adults, adolescents, and/or children (Huang et al. 2017, 2020a, 2020b; Kim et al. 2017a, 2017b; Meeker and Ferguson 2011; Park et al. 2017; Souter et al. 2020a; Weng et al. 2017) in some studies; other studies did not observe this association in adults (Dirtu et al. 2013; Meeker et al. 2007) or adolescents or children (Boas et al. 2010; Huang et al. 2017; Meeker and Ferguson 2011).

Animal Studies—Thyroid/Parathyroid Gland. A limited number of animal studies were found in the literature that evaluated the function of the thyroid gland; findings were inconsistent between studies. Increased serum total T3 and total T4 and hypothalamic thyrotropin-releasing hormone (TRH) were observed in adult Wistar rats exposed to 500 mg/kg/day for 4 weeks, but not ≤50 mg/kg/day (Sun et al. 2018). No changes were observed in free T3, free T4, or TSH. In contrast, free T4 and TSH levels were decreased in adult Sprague-Dawley rats exposed to 500 mg/kg/day for 31 days, with no change in free T3 or TRH (Zhang et al. 2018b). These findings were accompanied by microscopic and ultrastructural changes in the thyroid follicular cells, including cellular hypertrophy. Similarly, in weanling Sprague-Dawley rats exposed to DEHP for 30 days, changes in thyroid hormone levels included decreased serum total T3 and free and total T4 at ≥500 mg/kg/day and decreased free T3 and TRH at 750 mg/kg/day (Ye et al. 2017). Histological and ultrastructural changes in thyroid follicular cells were observed at ≥250 and 750 mg/kg/day, respectively. Following gestational and lactational exposure, decreased serum total T4 and increased serum TSH were observed in PND 7 and 14 Wistar rat offspring at maternal doses ≥30 mg/kg/day (Dong et al. 2019). When exposure continued through PND 21, serum thyroid hormone changes were only observed at ≥300 mg/kg/day. Ultrastructural changes in thyroid follicular cells were observed at ≥30 mg/kg/day at all timepoints. However, in a second rat developmental study, there were no changes in serum thyroid hormones in PND 21 or 63 offspring born to Sprague-Dawley dams exposed to DEHP at doses up to 400 mg/kg/day from GD 6 to PND 20 (Kobayashi et al. 2006). In adult C57BL/6 male mice, an increase in serum T4 was also observed after exposure to ≥100 mg/kg/day for 35 days; no other thyroid hormones were evaluated, and this finding was not observed at ≤10 mg/kg/day (Li et al. 2018). In adult ICR mice, no changes in serum T3 or T4 were observed following a 28-day exposure to doses up to 400 mg/kg/day (Xu et al. 2019).

One gavage study reported thyroid hyperplasia and hypertrophy in Sprague-Dawley rats exposed to 150 mg/kg/day for 13 weeks starting on PND 6 (Kim et al. 2018c). Increases in thyroid cell proliferation

were also observed in males and females at 150 and ≥30 mg/kg/day, respectively. The relative contributions of developmental and post-sexual maturation exposure on thyroid histology in this study are unknown. No changes in thyroid/parathyroid weight were observed. No changes in thyroid/parathyroid weight or histology were observed in any other oral study reviewed. In rats, no exposure-related weight and/or histology effects were observed in acute- or intermediate-duration studies at doses up to 3,000 mg/kg/day (Astill et al. 1986; Gray et al. 1977; Myers 1992b; NTP 1982; Poon et al. 1997), chronic-duration studies at doses up to 939 mg/kg/day (David et al. 2000a; Kluwe et al. 1982a, 1985; NTP 1982), gestational/lactation exposure studies at doses up to 405 mg/kg/day (Grande et al. 2006), or 2- or 3-generation studies at doses up to 659 mg/kg/day (Blystone et al. 2010; NTP 2005; Voss et al. 2005). In mice, no exposure-related weight and/or histology effects were observed in intermediate-duration studies at doses up to 7,899 (Myers 1992a; NTP 1982; Toyosawa et al. 2001) or chronic-duration studies at doses up to 1,821 mg/kg/day (David et al. 2000a; Kluwe et al. 1982a; NTP 1982). In other species, no exposure-related weight and/or histology effects were observed in sexually immature Cynomolgus monkeys exposed to 500 mg/kg/day for 14 days (Pugh et al. 2000), marmoset monkeys following at doses up to 2,500 mg/kg/day for 13 weeks (Kurata et al. 1998), ferrets exposed to 1,200 mg/kg/day for 14 months (Lake et al. 1976), or dogs exposed to 56.6 mg/kg/day for 1 year (Carpenter et al. 1953).

Mechanism of Thyroid Disruption. Several mechanisms have been proposed for phthalate-induced disruption in thyroid homeostasis (Dong et al. 2017; Kim et al. 2018c, 2019a; Li et al. 2020; Miodovnik et al. 2014). Transcriptional activity of the sodium-iodine symporter (NIS) was altered by some phthalates, resulting in reduced uptake of iodine into the thyroid (Miodovnik et al. 2014). DEHP was shown to be a thyroid receptor antagonist, and it inhibited the binding of T3 to the purified thyroid hormone receptor (Miodovnik et al. 2014) and T4 to integrin $\alpha_v\beta_3$, a plasma membrane bound thyroid hormone receptor (Li et al. 2020). DEHP can also increase the metabolism of thyroid hormones and interfere with thyroid hormone binding proteins (Kim et al. 2019a). Dong et al. (2017) analyzed gene and protein expression in the thyroid, pituitary, and hypothalamus of rats exposed chronically to DEHP and the results suggested a disruption of the hypothalamus-pituitary-thyroid axis through altered TSH/TSH receptor signaling. Altered expression of the TSH receptor in the hypothalamus and the TRH receptor in the pituitary were also observed in the DEHP-exposed rats (Ye et al. 2017; Zhang et al. 2018b). Kim et al. (2018c, 2019a) also observed thyroid cell proliferation and gene expression changes consistent with altered thyroid hormone regulation following *in vivo* or *in vitro* exposure. Ye et al. (2017) also proposed that observed oxidative stress and altered expression of enzymes in the liver may contribute to the downregulation of thyroid hormones.

Animal Studies—Pancreas. As discussed in Section 2.6 (Gastrointestinal), pseudoductular lesions and altered acinar cell foci were observed in the pancreas of rats administered dietary DEHP at 1,600 mg/kg/day for 108 weeks (only dose tested) (Rao et al. 1990). These lesions are expected to affect digestive system (exocrine) functions of the pancreas, as opposed to endocrine function. No other chronic-duration studies reported histopathological lesions in the pancreas in dogs at 56.6 mg/kg/day (Carpenter et al. 1953), rats at doses up to 939 mg/kg/day (Carpenter et al. 1953; David et al. 2000a; Kluwe et al. 1982a, 1985; NTP 1982), or mice at doses up to 1,821 mg/kg/day (David et al. 2000b; Kluwe et al. 1982a; NTP 1982). Similarly, no histopathological lesions in the pancreas were observed following intermediate-duration exposure to doses up to 2,500 mg/kg/day in monkeys (Kurata et al. 1998), 3,000 mg/kg/day in rats (Gray et al. 1977; Myers 1992b; NTP 1982; Poon et al. 1997), or 7,899 mg/kg/day in mice (Myers 1992a; NTP 1982; Toyosawa et al. 2001).

Epidemiology Studies—Adrenal Function. One pregnancy cohort study with 553 mother-child pairs evaluated potential associations between maternal DEHP exposure and cord blood cortisol and cortisone levels (Sun et al. 2018). Increased first and third trimester urinary MEHP levels were associated with increased cortisone cord levels in girl infants only. No association between cord blood cortisone levels were observed in either sex for MECPP, MEHHP, MEOHP, or ΣDEHP levels during any trimester. Increased third trimester urinary MEHP, MEHHP, MEOHP, and ΣDEHP levels were associated with increased cord blood cortisol levels in female infants. In contrast, increased third trimester urinary MECPP, MEHHP, and MEOHP levels were associated with decreased cord blood cortisol levels in male infants. No associations between cord blood cortisol and first or second trimester DEHP urinary metabolite levels. No other studies evaluating adrenal function and DEHP exposure in humans were identified.

Animal Studies—Adrenal Gland. The function of the adrenal gland was evaluated in developmental studies and reported an approximate 50% reduction in serum aldosterone levels in male adult offspring of Sprague-Dawley rats exposed to DEHP at doses ≥100 mg/kg/day from GD 14 to PND 0 (Martinez-Arguelles et al. 2011, 2013). In female offspring, serum aldosterone was significantly increased by approximately 2-fold at maternal doses of 300 mg/kg/day (Martinez-Arguelles et al. 2011). These changes were not observed in PND 21 offspring. No changes in serum corticosterone were observed in either sex at either time point at maternal doses up to 750 mg/kg/day (Martinez-Arguelles et al. 2011). While no changes were observed in serum angiotensin levels (which stimulate aldosterone production), significant reductions in angiotensin receptors *Agtr1a*, *Agtr1b*, and *Agtr2* were observed in the adrenal

gland of adult male offspring of DEHP-exposed dams (not assessed in female offspring) (Martinez-Arguelles et al. 2011).

Histopathological changes in the adrenal gland were observed inconsistently in oral studies in adult F344 rat. In a 3-generation study of F344 rats, adrenal cortical vacuolation was observed in F0 male rats exposed to a dietary dose of approximately 659 mg/kg/day, but not at doses ≤447 mg/kg/day (Blystone et al. 2010; NTP 2005). This was not observed in F1 or F2 parental males or parental females from any generation (Blystone et al. 2010; NTP 2005). Increased vacuolation and width in the zona glomerulosa in the adrenal gland were also observed in male and female F344 rats exposed to dietary doses ≥1,724 mg/kg/day for 13 weeks; no histopathological changes were observed at doses ≤918.4 mg/kg/day (Myers 1992b). However, no changes in adrenal histology were reported in F344 rats following dietary exposures up to 3,000 mg/kg/day for 12 weeks or 774 mg/kg/day for 2 years (Kluwe et al. 1982a, 1985; NTP 1982).

In other rat strains (Sprague-Dawley, Wistar, Sherman), no histopathological changes were observed in the adrenal glands in intermediate-duration studies at doses up to 10,000 mg/kg/day (Dalgaard et al. 2000; Poon et al. 1997), in chronic-duration studies at doses up to 300 mg/kg/day (Carpenter et al. 1953; Voss et al. 2005), or in a 2-generation study at doses up to 1,088 mg/kg/day (Schilling et al. 2001). Additionally, no changes in adrenal histology were observed in Wistar rats following intermittent nose-only inhalation concentrations up to 63 ppm for 4 weeks (Klimisch et al. 1992). In mice, no changes in adrenal gland histology were observed in intermediate-duration studies at doses up to 7,899 mg/kg/day (Myers 1992a; NTP 1982; Toyosawa et al. 2001; Xu et al. 2019) or chronic-duration studies at doses up to1,821 mg/kg/day for 2 years (Kluwe et al. 1982a; NTP 1982). In other mammalian species, no changes in adrenal gland histology were observed in marmoset monkeys following exposure to gavage doses up to 2,500 mg/kg/day for 13 weeks (Kurata et al. 1998), ferrets exposed to 1,200 mg/kg/day for 14 months (Lake et al. 1976), or dogs exposed to 56.6 mg/kg/day for 1 year (Carpenter et al. 1953)

Studies of adrenal gland weight following oral DEHP exposure during early life stages do not indicate a consistent organ weight effect attributable to exposure. Decreased adrenal weight was observed in adult offspring of Sprague-Dawley rats exposed to 750 mg/kg/day from GD 14 to PND 0, but not ≤300 mg/kg/day (Martinez-Arguelles et al. 2011). In a series of experiments in Sprague-Dawley and Long-Evans weanling male rats, adrenal gland weight was significantly decreased in Sprague-Dawley rats exposed to ≥100 mg/kg/day for 22 days immediately following weaning, but not following exposures to up to 900 mg/kg/day for 35, 42, or 76 days postweaning (Noriega et al. 2009). In Long-Evans rats,

adrenal gland weight was significantly decreased at 900 mg/kg/day, but not ≤300 mg/kg/day, following exposure for 35 days post-weaning, but not following exposure for 42 or 76 days (22-day duration not examined in Long-Evans rats) (Noriega et al. 2009). The study authors did not propose a rationale for why adrenal gland weight effects disappeared with longer exposure duration, but it may represent a transient effect to initial exposure that recovers with time. Male offspring of Wistar rats exposed to DEHP at doses ≥10 mg/kg/day from GD 7 to PND 16 also showed decreased adrenal weight on PND 16 in one study, but not at doses up to 100 mg/kg/day in another using the same protocol (Christiansen et al. 2010). No change in adrenal gland weight was observed in male Wistar rats exposed to DEHP on PNDs 1–21 (via dam) or PNDs 22–52 (direct) at doses up to 75 mg/kg/day (Venturelli et al. 2015), or from GD 13 to PND 21 (via dam) at doses up to 700 mg/kg/day (Venturelli et al. 2019).

In contrast, *increased* relative adrenal gland weights were observed in F0, F1, and F2 parental male rats exposed to an approximate dietary dose of 659 mg/kg/day, but not ≤447 mg/kg/day, during a 3-generation reproductive study (Blystone et al. 2010; NTP 2005). Adrenal weight changes were not observed in parental females. Increased absolute adrenal gland weight (without a change in body weight) was also reported in male mice following gavage exposure to 400 mg/kg/day for 28 days (Xu et al. 2019). No exposure-related changes in adrenal gland weight were reported in any other oral study in rats reviewed, including acute-duration studies with doses up to 5,000 mg/kg/day (Berman et al. 1995; Lee and Koo 2007), intermediate-duration studies with doses up to 10,000 mg/kg/day (Dalgaard et al. 2000; Gray et al. 1977), a lifetime exposure study with doses up to 300 mg/kg/day (Voss et al. 2005), a 2-generation study with doses up to 1,088 mg/kg/day (Schilling et al. 2001), or a developmental study with doses up to 300 mg/kg/day (Gray et al. 2009). Similarly, no change in adrenal weight was observed in a 4-week inhalation study in rats at nose-only concentrations up to 63 ppm (Klimisch et al. 1992). In sexually immature Cynomolgus monkeys, no exposure-related changes in adrenal weight were observed following gavage exposure to 500 mg/kg/day for 14 days (Pugh et al. 2000).

Gestational exposure to DEHP produced effects on the adrenals of adult offspring, including altered control of aldosterone and changes to cholesterol and lipid metabolism (Lee et al. 2017; Martinez-Arguelles and Papadopoulos 2015; Martinez-Arguelles et al. 2013). DEHP exposure *in utero* resulted in decreased adrenal aldosterone production and decreased mineralocorticoid receptor (MR) expression in adult Leydig cells (at PND 60, but not PND 21), leading to reduced testicular testosterone formation independent of a direct effect on the steroidogenic pathway. Cortisone levels were not affected, suggesting that DEHP induced alterations in fetal zona glomerulosa development. In isolated glomerulosa cells, DEHP increased many of the same genes upregulated by angiotensin II and potassium,

including genes encoding potassium channels, at PND 60 but not PND 21 (Martinez-Arguelles et al. 2013). The PPARα pathways appear to be critical for maintaining adequate aldosterone biosynthesis in the adult rat.

DEHP was shown to interfere with mitochondrial cholesterol transport in *ex vivo* zona glomerulosa cells obtained from PND 20 rats exposed to 500 mg/kg DEHP for 10 days. Global gene expression data showed down-regulation of the gene encoding hormone-sensitive lipase (*Lipe*) and a decrease in the levels of free cholesterol available for steroid biosynthesis at PND 60 (male rats exposed *in utero*) (Martinez-Arguelles and Papadopoulos 2015; Martinez-Arguelles et al. 2013).

Animal Studies—Pituitary Gland. No exposure-related changes in serum adrenocorticotropin levels were observed in male or female adult offspring of Sprague-Dawley rats exposed to DEHP at doses ≥100 mg/kg/day from GD 14 to PND 0 (Martinez-Arguelles et al. 2011). No additional studies evaluating serum pituitary hormone levels were identified.

The incidence of vacuolation of basophils in the pars distalis in the pituitary gland was increased in male Sprague-Dawley rats after dietary exposure to DEHP at doses ≥737 mg/kg/day for 17 weeks; this effect was not observed in males exposed to 142 mg/kg/day or at 2- or 4-week interim sacrifices at doses up to 1,440 mg/kg/day (Gray et al. 1977). These cells are known as “castration cells” because they appear after gonadectomy due to decreased testosterone secretion by the testes, and are therefore considered a sensitive indicator of gonadal deficiency. Increased “castration cells” were also observed in male F344 rats in a 13-week study following dietary exposure to 1,724 mg/kg/day, but not ≤850.1 mg/kg/day (Myers 1992b) and in a 2-year study following dietary exposure to 789 mg/kg/day, but not ≤147 mg/kg/day (David et al. 2000a). See Section 2.16 (Reproductive) for more information regarding gonadal effects of DEHP exposure.

Hypertrophy of anterior pituitary cells (pars anterior) was observed in male F344 rats administered approximately 674 mg/kg/day for 2 years; no changes were observed at 322 mg/kg/day (Kluwe et al. 1982a, 1985; NTP 1982). No changes were observed in females at doses up to 774 mg/kg/day. Anterior pituitary cell hypertrophy was not observed in other chronic-duration F344 rat study at doses up to 939 mg/kg/day (David et al. 2000a), or shorter-duration studies in F344, Sprague-Dawley, or Wistar rats at doses up to 3,000 mg/kg/day (Blystone et al. 2010; Gray et al. 1977; Myers 1992b; NTP 1982, 2005; Poon et al. 1997; Schilling et al. 1999, 2001). DEHP was shown to down-regulate the expression of

estrogen receptor (ER) α and β in primary cultures of rat anterior pituitary cells, which may be related to altered pituitary cell growth (Perez et al. 2020).

In mice, no histopathological changes in the pituitary gland were observed following intermediate-duration exposure to doses up to 7,899 mg/kg/day (Myers 1992a; NTP 1982; Toyosawa et al. 2001) or chronic-duration exposure to doses up to 1,821 mg/kg/day (David et al. 2000a; Kluwe et al. 1982a; NTP 1982). In nonhuman primates, no histopathological changes in the pituitary gland were observed in marmoset monkeys following exposure to doses up to 2,500 mg/kg/day for 13 weeks (Kurata et al. 1998).

No exposure-related changes were observed in pituitary weights of Sprague-Dawley rats exposed to doses up to 1,440 mg/kg/day for 17 weeks (Gray et al. 1977), or F0 or F1 Wistar rats exposed to doses up to 1,088 mg/kg/day over 2 generations (Schilling et al. 1999, 2001), or marmoset monkeys exposed to doses up to 2,500 mg/kg/day for 13 weeks (Kurata et al. 1998).

Summary. Data from epidemiological studies suggest that there may be a possible association between DEHP exposure and altered thyroid hormone levels in humans, although the individual studies have additional limitations not described in detail here (e.g., cross-sectional design, small sample size, lack of consistent control for potential confounders). There were no consistent alterations in thyroid function following DEHP exposure from the limited number of available animal studies. In animals, there is some evidence for adverse effects in the adrenal and pituitary glands. Animal data suggest that the developing animal may be particularly sensitive to DEHP-mediated effects in endocrine organs.

2.14 IMMUNOLOGICAL

Overview. Epidemiological data on immune system effects of DEHP include studies addressing potential associations between prenatal DEHP exposure and asthma, wheezing, elevated IgE, eczema, atopic dermatitis, and food allergy. Several animal studies evaluated the potential for DEHP exposure via inhalation or oral exposure to enhance allergic immune reactions in sensitized animals. Additional animal studies evaluated immune organ weight and histology. Potential underlying mechanisms for the observed adjuvant effect have also been studied.

Epidemiology Studies. Epidemiological studies of immunological health outcomes (including allergy, asthma, serum IgE levels, etc.) selected for review are in Table 2-7. In a cohort study that examined the risk for asthma symptoms and wheezing, Gascon et al. (2015a) reported increased risk of wheeze between birth and age 7 and risk of asthma at age 7 with doubling of maternal DEHP metabolite levels in urine.

Table 2-7. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Araki et al. 2020 Cross-sectional, 128 children (68 boys, 60 girls; age 7–12 years), Japan	Wheeze, rhinoconjunctivitis, or eczema	ΣDEHP (MEHP, MEOHP, MECPP)	IQR: 179–512 nmol/g Cr	↔
Ashley-Martin et al. 2015 Cohort, 1,137 children (maternal urine evaluated), Canada	IgE in cord blood	ΣDEHP	NR	↔
		MEHP	IgE ≥0.5 ku/L: GM (GSD): 2.6 (2.7) ng/mL IgE <0.5 ku/L: 2.6 (2.5)	NR
		MEHHP	IgE ≥0.5 ku/L: 10.4 (2.7) IgE <0.5 ku/L: 10.6 (2.4)	NR
		MEOHP	IgE ≥0.5 ku/L: 7.4 (2.5) IgE <0.5 ku/L: 7.4 (2.3)	NR
	IL-33 and TSLP in cord blood	ΣDEHP	NR	↔
		MEHP	IL-33 and TSLP ≥80th percentile: 2.5 (2.6) IL-33 and TSLP <80th percentile: 2.7 (2.5)	NR
		MEHHP	IL-33 and TSLP ≥80th percentile: 9.4 (2.6) IL-33 and TSLP <80th percentile: 10.7 (2.5)	NR
		MEOHP	IL-33 and TSLP ≥80th percentile: 6.8 (2.5) IL-33 and TSLP <80th percentile: 7.5 (2.3)	NR
Bekö et al. 2015 Case-control, 200 cases, children (age 3–5 years) with at least two conditions (asthma, allergic rhinoconjunctivitis, or eczema), and 300 controls, Denmark	**IgE sensitization**	MEHP	Cases: IgE-: Median: 3.7 ng/mL; IgE+: 4.01 Controls: 5.18	↔
		MEHHP	Cases: IgE-: 31.7; IgE+: 33.2 Controls: 33.5	↔
		MEOHP	Cases: IgE-: 13.3; IgE+: 16.0 Controls: 17.5	↔
		MECPP	Cases: IgE-: 29.9; IgE+: 31.5 Controls: 36.6	↑
		IgE sensitization was associated with MECPP only among asthma patients; no associations between DEHP metabolites among controls or among cases with rhinoconjunctivitis and atopic dermatitis.		

Table 2-7. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Bertelsen et al. 2013 Cross-sectional, 623 children (age 10 years), Norway	Asthma	ΣDEHP (MEP, MEHHP, MEOHP, MECPP)	IQR: 0.58–1.18 µmol/L (SG-adj)	↔
Franken et al. 2017 Cross-sectional, 418 adolescents (mean age 14.8 years), Belgium	**Asthma**	**ΣDEHP** (MEHP, MEHHP, MEOHP)	IQR: 0.07–0.22 µmol/L (SG-adj)	↑
Gascon et al. 2015a Cohort, 391 children, allergy outcomes determined at age 6 and 14 months and 4 and 7 years, maternal urine evaluated, Spain	**Wheeze or asthma (age 7 years)**	**ΣDEHP** (MEHP, MEHHP, MEOHP, MECPP)	IQR: 69.5–147.9 µg/g Cr	↑
	Eczema (age 7 years) or atopy (age 4 years)	ΣDEHP	See above	↔
Hoppin et al. 2013 Cross-sectional, 2,325 children (age ≥6 years) and adults (age ≥18 years), United States (NHANES)	Allergic symptoms	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	Children: IQR: 54.15–230.02 ng/mL (survey-weighted) Adults: 33.21–160.81	↔
Hsu et al. 2012 Cross-sectional, 101 children (mean age 7 years), Taiwan	Asthma, rhinitis, or eczema	MEHP	IQR: 5.7–20.0 µg/g Cr	↔
Johnk et al. 2020 Cohort, 552 mother-child pairs (age 5 years), maternal urine evaluated, Denmark	Wheeze, asthma, eczema, or rhinitis in children	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	IQR: 9.4–34.6 ng/mL	↔

Table 2-7. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Just et al. 2012 Cohort, 244 children, fractional exhaled NO concentrations (FeNO; a marker of airway inflammation) measured at age 4.9–9.1 years (maternal urine evaluated), United States (New York)	FeNO	MEHHP	GM (95% CI): 42 (36, 49) ng/mL	↔
Kim et al. 2018e Cross-sectional 56 children with asthma; age 6–16 years, Korea	**FeNO**	**MEHHP**	IQR: 35.2–85.7 µg/g Cr	↑
		MEOHP	27.2–60.6	↑
Ku et al. 2015 Cohort, 171 children evaluated for asthma symptoms (age 8 years; maternal and child urine evaluated), Taiwan	Wheezing or asthma	ΣDEHP (MEHP, MEHHP)	Maternal: GM (95% CI): 50.22 (42.22, 59.72) µg/g Cr	↔
	Serum IgE (in allergic children)	ΣDEHP	Maternal: see above	↔
			Child (5 years): NR	↔
		MEHP	Maternal: 16.90 (14.49, 19.72)	↑
			Child (5 years): GM: 11.9 µg/g Cr	↑
	Serum IgE (in non-allergic and all children)	ΣDEHP or MEHP	Maternal: see above	↔
			Child (5 years): see above	↔
Lin et al. 2018 Cohort, 191 children (115 with allergic disease, 76 without allergic disease), children assessed for allergy at mean age 9.2 years (urine collected at age 2, 5, and 9 years), Taiwan	Asthma, allergic rhinitis, atopic dermatitis, elevated IgE, or serum IgE	MEHP	Child (2 years): GM: 38.3 µg/g Cr	↔
			5 years: 14.8	↔
			9 years: 3.7	↔
		Metabolite concentrations were estimated from graphically presented data using GrabIt! software.		

Table 2-7. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Podlecka et al. 2020 Cohort, 145 mother-child pairs, children assessed for allergy at age 9 years, Poland Maternal and 2-year-old urine levels obtained from previous publication (Polanska et al. 2014)	**Food allergy**	MEHP	Maternal: median (range): 0.2 (0.02–3.5) ng/mL Child (2 years): 0.02 (0.02–176) Child (9 years): 3.04 (NR)	↔
		MEHHP	Maternal: 2.0 (0.05–256) Child (2 years): 2.1 (0.05–190) Child (9 years): 20.34 (NR)	↔
		MEOHP	Maternal: 1.3 (0.05–132) Child (2 years): 1.2 (0.05–283) Child (9 years): 8.03 (NR)	↑
	Atopic dermatitis	MEHP or MEOHP	See above	↔
		MEHHP	See above	↑
	Allergic rhinitis, or Asthma and Wheezing	MEHP, MEHHP, or MEOHP	See above	↔
Stelmach et al. 2015 Cohort, 147 children (age 2 years), maternal and child urine evaluated, Poland	Atopic dermatitis or food allergy	ΣDEHP	Maternal: IQR: 1.73–37.75 µg/g Cr Child (2 years): 1.81–9.11	↔
		MEHP	Maternal: 0.04–0.64 Child: 0.02–0.02	↔
		MEHHP	Maternal: 0.11–20.57 Child: 1.09–5.37	↔
		MEOHP	Maternal: 0.69–6.54 Child: 0.48–2.79	↔
Strassle et al. 2018 Cross-sectional, 1,091 adults age ≥18 years (mean age 44.5 years), United States (NHANES)	Wheeze, asthma, rhinitis, or hay fever	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	IQR: 34.30–177.10 ng/mL	↔
		Wheeze was associated with increased ΣDEHP individuals with high exposure to house dust endotoxin (≥25 EU/mg)		

Table 2-7. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Wang et al. 2014 Cohort/Cross-sectional, 483 children (244 boys and 239 girls), atopic disorders determined at ages 2 and 5 years, (child's urine evaluated at ages 2 and 5 years), Taiwan	**Serum IgE**	**MEHP**	GM (SE): 16.01 (1.12) µg/g Cr	**All:** ↑ **Boys:** ↑ Girls: ↔
	Atopic dermatitis	MEHP	See above	↔
Whyatt et al. 2014 Cohort, 300 children, asthma determined when children were age 5, 6, 7, 9, and 11 years (maternal urine evaluated), United States (New York)	Current and/or history of asthma symptoms	MEHHP	IQR: 10.6–50.0 ng/mL	↔

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; FeNO = fractional exhaled NO concentration; GM = geometric mean; GSD = geometric standard deviation; IgE = immunoglobulin E; IL-33 = interleukin 33; IQR = interquartile range; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl) phthalate; NHANES = National Health and Nutrition Examination Survey; NR = not reported; SE = standard error; SG-adj = specific gravity adjusted; TSLP = thymic stromal lymphopoietin

However, DEHP metabolites were not associated with asthma symptoms or wheezing in children in the other cohort studies (Johnk et al. 2020; Ku et al. 2015; Lin et al. 2018; Podlecka et al. 2020; Whyatt et al. 2014). Maternal urinary MEHHP levels during pregnancy were not associated with a change in fractional exhaled nitric oxide (FeNO, a marker of airway inflammation) in children ~5–9 years old (Just et al. 2012); other metabolites of DEHP were not measured in this study. In a cross-sectional study, Franken et al. (2017) reported increased risk of asthma in adolescents with an interquartile increase in the sum of DEHP urinary metabolites. No associations were observed between DEHP exposure and asthma symptoms or wheezing in cross-sectional studies in children (Araki et al. 2020; Bertelsen et al. 2013; Hsu et al. 2012) or adults (Strassle et al. 2018). However, urinary MEHHP and MEOHP levels were associated with FeNo levels in a cross-sectional analysis of children and adolescents with asthma (Kim et al. 2018e).

One cohort study reports increased risk of food allergy at age 9 years with increased maternal or child urinary levels of MEOHP (but not MEHP or MEHHP) and increased risk of atopic dermatitis at age 9 years with increased maternal or child urinary levels of MEHHP (but not MEHP or MEOHP) (Podlecka et al. 2020). No association with allergic rhinitis was observed. In other studies, no associations between DEHP urinary metabolites and food allergy, eczema, atopy, rhinitis, hay fever, and/or general allergic symptoms were observed in cohort studies in children (Gascon et al. 2015a; Johnk et al. 2020; Lin et al. 2018; Stelmach et al. 2015; Wang et al. 2014) or cross-sectional studies in children (Araki et al. 2020; Hoppin et al. 2013; Hsu et al. 2012) or adults (Strassle et al. 2018). No association was observed between DEHP metabolites in maternal urine during pregnancy and cord blood levels of interleukin-33 (IL-33) or thymic stromal lymphopoietin (TSLP), inflammatory markers that, when elevated in cord blood, predict allergic disease later in life (Ashley-Martin et al. 2015).

Maternal levels of DEHP urinary metabolites were not associated with IgE in cord blood (Ashley-Martin et al. 2015). However, MEHP levels in both maternal urine (during pregnancy) and children's urine at 5 years of age were positively associated with higher serum IgE in children 8 years of age that were diagnosed with allergic symptoms (Ku et al. 2015). No association was observed in non-allergic children, or when allergic and non-allergic children were combined. In another cohort of 9-year-old children, serum IgE levels were not associated with urinary MEHP levels measured at 2, 5, or 9 years of age; no other DEHP metabolites were evaluated (Lin et al. 2018). A cross-sectional study of children 3–5 years of age did not find an association between the children's MEHP, MEHHP, or MEOHP urinary levels and IgE sensitization (Bekö et al. 2015), although urinary MECPP was associated with IgE sensitization.

Interestingly, Wang et al. (2014) reported that only 2-year-old boys had urinary MEHP levels positively associated with serum IgE, although girls were also evaluated.

One cross-sectional study evaluated potential associations between DEHP exposure and markers of autoimmunity in adult women (Souter et al. 2020a). Urinary DEHP metabolite levels were not associated with thyroid peroxidase or thyroglobulin antibodies.

Animal Studies—Immune Function. Several animal studies have reported adjuvant effects of low levels of DEHP exposure in rodents sensitized to OVA. In these studies, OVA-sensitized rodents were exposed to DEHP prior to an OVA challenge. Immune responses were measured in treated animals and compared with responses in OVA-sensitized controls. The human health relevance of findings in these sensitized animals is uncertain in the absence of clear evidence that the immune system is a target of DEHP toxicity in humans or unsensitized animals.

In an inhalation study, OVA-sensitized mice intermittently exposed to 0.81 ppm DEHP for 14 weeks showed elevated OVA-specific IgG1, eosinophils, neutrophils, and lymphocytes following a 3-day OVA challenge (Larsen et al. 2007). Immune responses were not elevated at exposure concentrations ≤0.11 ppm. This study did not evaluate non-sensitized animals.

Enhanced immune responses in OVA-sensitized rodents were also observed following oral exposure to DEHP. The lowest oral dose associated with an altered immune response was 0.03 mg/kg/day based on increased OVA-specific IgE and IgG after 28 days (Han et al. 2014a) or total serum IgE after 52 days of exposure (Guo et al. 2012). More consistent evidence for enhanced immune responses was observed in these studies at 3 mg/kg/day, including increased cytokine production, germinal center formation in splenic lymphoid nodules, altered T-cell subpopulations, increased eosinophils in BAL fluid, and airway remodeling. Yang et al. (2008) reported similar enhancements in the immune response of OVA-sensitized mice after DEHP exposure to ≥0.7 mg/kg/day (lowest dose tested) for 30 days.

Increased airway hyperresponsiveness was also reported in both sensitized and non-sensitized animals exposed to ≥0.7 and 70 mg/kg/day, respectively, compared with appropriate controls (Yang et al. 2008). However, the magnitude of effect was greater in sensitized animals. Similarly, a limited number of endpoints were altered in non-sensitized animals exposed to 3 mg/kg/day for 52 days, including elevated anti-OVA-IgE levels and lung tissue IFN-γ (Guo et al. 2012). Han et al. (2014a) did not evaluate non-sensitized animals.

Developmental exposure to DEHP also enhanced immune responses in OVA-sensitized rats following gestational and lactational exposure to oral doses ≥30 mg/kg/day (lowest dose tested) (Wang et al. 2018). Immune changes following OVA challenge were increased, including OVA-specific serum IgE and IgG1, cytokine production, and follicular helper cell population. Wang et al. (2018) also observed increases in the severity of tissue cell infiltration, airway remodeling, and germinal center formation in splenic lymphoid nodules at ≥0.3 mg/kg/day. Increases in eosinophils in BAL fluid and airway responsiveness were observed at maternal doses ≥30 mg/kg/day. No exposure-related findings were observed in non-sensitized animals.

Similar adjuvant responses were not observed in studies using other allergens. For example, intermittent oral exposure to DEHP at doses up to 19 mg/kg/day (1 day/week for 4 weeks) did not increase allergen-induced atopic dermatitis in mice exposed to the mite allergen (*Dermatophagoides pteronyssinus*), compared with allergen-only exposed controls (Sadakane et al. 2014). Similarly, delayed-type hypersensitivity (DTH) responses to keyhole limpet hemocyanin (KLH) were not increased in female rats following a 16-day exposure to DEHP at concentrations up to 300 mg/kg/day (Piepenbrink et al. 2005). In this study, rats were sensitized to KLH at 11 and 12 weeks post-exposure and evaluated for DTH responses 13 weeks post-exposure. Piepenbrink et al. (2005) also evaluated DTH responses in juvenile and adult female offspring of rats exposed to DEHP at doses up to 300 mg/kg/day from GD 6 to 21. As seen in exposed adults, enhanced DTH responses were not observed following developmental exposure. Cytokine levels were elevated in non-sensitized, non-challenged Sprague-Dawley rats and C57BL/6J mice following drinking water exposure to doses ≥300 mg/kg/day for 30 days (Wang et al. 2020). In Sprague-Dawley rats, levels of IL-12, TNF-α, IFN-γ, and IL-2 were elevated; in C57BL/6J mice, levels of IL-1, IL-6, and monocyte chemoattractant protein-1 (MCP-1) were elevated. No changes in cytokine levels were observed in similarly exposed Wistar rats or BALB/c mice at doses up to 3,000 mg/kg/day.

There is some evidence of altered immune endpoints measured *ex vivo* following DEHP exposure. In the inhalation study described above, mediastinal lymph nodes harvested from treated OVA-sensitized animals had significantly increased *ex vivo* secretion of the cytokines IL-5 and IL-10, compared with lymph nodes harvested from OVA controls (Larsen et al. 2007). However, evaluation of splenic immune function *ex vivo* has not shown exposure-related immune alterations following oral exposure to DEHP. No changes, compared with controls, were observed in mitogenesis in spleen cells harvested from mice exposed to DEHP at dietary doses up to 360 mg/kg/day for 10 or 20 days (Sasaki et al. 2003). Similarly, in the Piepenbrink et al. (2005) study described above, no exposure-related changes were observed in *ex*

vivo cytokine production (interleukins [IL]-2, -4, -10, -12, or interferon [IFN]-γ) or production of signaling molecules TNF-α or nitric oxide by macrophages following in utero or adult exposure.

Animal Studies—Immune Organ Weight and Histology. One study reported thymic atrophy in mice exposed to ≥6,922 mg/kg/day for 28 days; no changes occurred at doses ≤2,579 mg/kg/day (Myers 1992a). No changes in thymic histology were observed in other mouse studies utilizing lower doses, including acute-duration studies at doses up to 360 mg/kg/day (Sasaki et al. 2003), intermediate-duration studies at doses up to 2,600 mg/kg/day (NTP 1982; Toyosawa et al. 2001; Xu et al. 2019), or chronic-duration studies at doses up to 1,821 mg/kg/day (Kluwe et al. 1982a, 1982b, 1985; NTP 1982). In rats, no changes in thymus histology were observed in acute studies at doses up to 5,000 mg/kg/day (Berman et al. 1995), intermediate-duration studies at doses up to 3,000 mg/kg/day (Gray et al. 1977; Myers 1992b; NTP 1982; Piepenbrink et al. 2005), chronic-duration studies at doses up to 774 mg/kg/day (Kluwe et al. 1982a, 1982b, 1985; NTP 1982), or a 2-generation study at doses up to 1,088 mg/kg/day (Schilling et al. 2001). Increased thymus weight was reported in one mouse study following a 28-day exposure to DEHP at 400 mg/kg/day; however, the relevance of this finding in the absence of histopathological changes is unclear (Xu et al. 2019). In other rodent studies, no exposure-related changes in thymic weights were observed in acute studies at doses up to 5,000 mg/kg/day (Berman et al. 1995), intermediate-duration studies at doses up to 1,857.6 mg/kg/day (Myers 1992b; Piepenbrink et al. 2005), a 2-generation study at doses up to 1,088 mg/kg/day (Schilling et al. 2001), or a gestational/lactation exposure study at doses up to 405 mg/kg/day (Grande et al. 2006).

No adverse effects were observed in other immune organs (spleen, lymph nodes, bone marrow) in any of the oral studies reviewed. In nonhuman primates, no changes in spleen weights were observed in sexually immature Cynomolgus monkeys exposed to 500 mg/kg/day for 14 days (Pugh et al. 2000). Carpenter et al. (1953) reported no changes in spleen histology in dogs at 56.6 mg/kg/day for 1 year. In rodents, no changes in spleen, lymph node, and/or bone marrow histology or weights were observed in a large number of studies at acute doses up to 5,000 mg/kg/day, intermediate-duration doses up to 10,000 mg/kg/day, or chronic-duration doses up to 1,821 mg/kg/day (Table 2-2). In addition, no changes in spleen histology were observed in a 4-week inhalation (nose-only) study in rats at concentrations up to 63 ppm (Klimisch et al. 1992).

Mechanisms of Altered Immune Function. The adjuvant effect of DEHP appears to be related to an imbalance in the humoral immune response mediated by cytokines released from hyperfunctioning T follicular helper cells (CD4+ Th cell subset) (Han et al. 2014a, 2019). These cells synthesize excesses

of IL-21 and IL-4, which result in increased secretion of allergy-related IgE and IgG1. DEHP increased the expression of signaling lymphocytic activation molecule-associated protein (SAP) and transcription factors Bcl-6 and c-Maf in T follicular helper cells (Han et al. 2014a, 2019). DEHP also enhanced the production and/or secretion of tumor necrosis factor-α (TNFα) by isolated macrophages or monocytes (Hansen et al. 2015). Direct activation of PPARs is not considered a likely mechanism for asthma, because PPARs primarily mediate anti-inflammatory effects in the lungs (Bolling et al. 2013).

Summary. Limited human data provide inconsistent findings, but some studies in sensitized animal suggest a potential association between DEHP exposure and enhanced immune system responses. One animal study reported thymic atrophy following high oral exposure; no additional studies evaluated this endpoint at comparable doses.

2.15 NEUROLOGICAL

Overview. Most of the epidemiological and animal data pertaining to neurological effects of DEHP are studies that have prenatal and/or early postnatal exposure; these studies are discussed in Section 2.17 (Developmental). One cohort evaluated depression in elderly subjects and five cross-sectional studies evaluated various neurological effects in adults using NHANES data. A limited number of oral studies in animals evaluated neurological function in adult animals following exposure to DEHP. Brain weight and nervous tissue histology were evaluated in one inhalation study and several oral studies in animals exposed to DEHP.

Epidemiology Studies. Shiue (2015a) observed no associations between urinary levels of DEHP metabolites and self-reported hearing difficulty among 5,560 adults (20–69 years of age) NHANES (2011–2012) participants. The frequency of self-reported memory problems over the previous 7 days was not associated with DEHP metabolite levels in 1,792 elderly adults (60–80 years old) participating in NHANES 2011–2012 (Shiue 2015b).

In a cohort of 535 elderly adults (mean age of 73 years), urinary MEHHP, MEOHP, MECPP, and ΣDEHP levels were associated with an increased score on the Korean version of the Short Form Geriatric Depression Scale, evaluated 1–3 times over a 2-year period (Lee et al. 2018). In a cross-sectional study, an analysis of 5,560 adult (20–80 years of age) NHANES (2011–2012) participants also observed an association between risk of depression and increased concentrations of MECPP in urine, but not other DEHP metabolites (Shiue 2015c). The association between prevalence of depression and MECPP levels

was sustained in a model that simultaneously accounted for concurrent health conditions (such as cardiovascular, neurological, respiratory, and digestive conditions, as well as other diseases) that could also increase the risk of depression (Shiue 2015c). However, due to the cross-sectional nature of the available data, coupled with uncertainty in how well urinary metabolite levels predict long-term exposure to DEHP, these findings are considered preliminary. In other cross-sectional studies, no association between prevalence of self-reported depression and urinary DEHP metabolites was reported in studies of 3,342 adults >18 years old participating in NHANES surveys between 2005 and 2008 (Berk et al. 2014) or 2,030 elderly adults (≥60 years) participating in NHANES surveys between 2005 and 2012 (Kim et al. 2016b).

Wang et al. (2015) reported clinical symptoms of neurotoxicity (i.e., headache, fatigue, dizziness, muscle weakness, nausea, and vomiting) in Chinese workers exposed to DEHP at three different PVC manufacturing facilities (average exposures ranging between 233 and 707 μg/m^3 DEHP in the three factories). As described in Section 2.9 (Hepatic), a correlation was observed between reduced plasma cholinesterase activity and DEHP residues in plasma. It is unclear whether the observed reduction in plasma cholinesterase activity is related to the reported clinical symptoms.

Animal Studies. No changes were observed in the histology of the brain, spinal cord, or sciatic nerve in rats following nose-only exposure to DEHP concentrations up to 63 ppm for 4 weeks (5 days/week, 6 hours/day) (Klimisch et al. 1992). Nervous system function was not assessed in this study, but no apparent clinical signs of toxicity were observed. No other studies regarding neurological effects in adult animals after inhalation exposure to DEHP were located.

A limited number of studies evaluated neurological function in adult rats after oral exposure to DEHP. A functional observational battery (FOB) and motor activity measurements were conducted in F344 rats before and after a single gavage dose of up to 5,000 mg DEHP/kg or daily gavage doses of up to 1,500 mg/kg/day for 10–14 days (Moser et al. 1995, 2003). The tests assessed autonomic, sensorimotor, and neuromuscular functions as well as excitability and activity. DEHP showed no neurobehavioral toxicity; however, a single administration of the 5,000 mg/kg dose produced signs of general debilitation (ptosis, piloerection, slight lacrimation, and hypothermia). Similarly, Dalgaard et al. (2000) did not observe exposure-related changes in FOB tests in rats at doses up to 10,000 mg/kg/day for 4 weeks or 1,000 mg/kg/day for 9 weeks. However, Liu et al. (2018b) reported elevated anxiety in rats in the elevated plus maze and open field testing following a 30-day exposure to 500 mg/kg/day; no changes in overall motor activity were observed. In the Morris water maze, rats exposed to ≥100 mg/kg/day for

5 months showed impaired spatial learning in the Morris water maze; no changes in swimming speed or spatial memory were observed at doses up to 500 mg/kg/day (Ran et al. 2019).

Similarly, a limited number of studies evaluated neurological function in adult mice after oral exposure to DEHP. No changes in exploratory behavior were observed in F0 mice from a 1-generation study after 3 weeks of exposure to doses up to 180.77 mg/kg/day (behavior assessed 1 week prior to mating) (Tanaka 2002). In contrast, another 3-week study in mice reported decreased swimming speed in the Morris water maze at ≥0.18 mg/kg/day and decreased total distance travelled in an open field at ≥18 mg/kg/day (Feng et al. 2020). Observed changes in motor activity did not appear to be secondary to anxiety since time spent in the center of the open field was increased at ≥1.8 mg/kg/day. There was also evidence of impaired learning and memory in the Morris water maze at ≥0.18 mg/kg/day (Feng et al. 2020); however, it is unclear if some or all of the observed effect was secondary to observed swimming impairments. At much higher doses (≥6,922 mg/kg/day), clinical signs of neurotoxicity were reported in mice exposed to DEHP for 28 days, including hunched posture in most animals and hypoactivity in a few animals (Myers 1992a). Tremors were observed in one female mouse prior to death at 7,899 mg/kg/day.

No exposure-related changes in brain, spinal cord, or peripheral nerve histology or brain weights were observed in any of the oral studies reviewed; however, studies other than those mentioned above did not assess neurological function. In nonhuman primates, no changes in brain weight occurred in marmoset monkeys exposed to 2,000 mg/kg/day for 14 days (ICI Americas Inc. 1982; Rhodes et al. 1986). In rodents, no changes in nervous system histology and/or brain weight were observed in numerous studies after acute-duration exposure to 1,100 mg/kg/day, intermediate-duration exposure to doses up to 10,000 mg/kg/day, or chronic-duration exposure to doses up to 1,821 mg/kg/day (Table 2-2). Additionally, no changes in brain histology were observed in ferrets exposed to 1,200 mg/kg/day for 14 months (Lake et al. 1976).

Summary. Human epidemiological data regarding neurological effects in adults are extremely limited. There is limited evidence of altered neurobehavior in rats and mice following exposure to low oral doses of DEHP.

2.16 REPRODUCTIVE

Overview. The potential effects of DEHP exposure on the male reproductive system have been evaluated in several human epidemiological studies, numerous rodent studies, and a limited number of studies in

nonhuman primates. Potential effects on the female reproductive system have been evaluated in humans and animals as well, but to a lesser extent. A large number of reproductive studies have focused on the potential effects of DEHP on the developing reproductive system following prenatal, early postnatal, and/or pre-pubescent exposure. These data are in Section 2.17 (Developmental). Data regarding reproductive system toxicity following exposure to DEHP in adult humans and in sexually mature animals are below. For studies that exposed animals both prior to and through sexual maturation into adulthood (e.g., multigenerational studies), endpoints evaluated prior to sexual maturation are in Section 2.17 (Developmental), while endpoints evaluated after sexual maturation are below. Several studies evaluating potential mechanisms of reproductive toxicity are also discussed.

Epidemiology Studies—Male Reproductive Effects. Data following inhalation exposure are limited to three small occupational studies in PVC workers evaluating serum hormone levels (Table 2-8) or sperm parameters (see Table 2-9). A study from China reported decreased free testosterone levels with increasing urinary MEHP levels in male workers (n=74); no other metabolites were evaluated and no associations were observed with serum estradiol, luteinizing hormone (LH), or follicle stimulating hormone (FSH) (Pan et al. 2006). A similar study in Taiwan (n=82) did not observe associations between DEHP urinary metabolites and total testosterone, estradiol, LH, FSH, inhibin B, or sex hormone-binding globulin (SHBG); free testosterone was not evaluated (Fong et al. 2015). In another Taiwanese study including 47 PVC workers and 15 controls, decreased sperm motility was associated with increased urinary MEHP, MEHHP, and MEOHP levels; no association was observed for sperm concentration or morphology (Huang et al. 2014a).

Cross-sectional studies evaluating potential associations between serum reproductive hormone and non-occupational DEHP exposure are presented in Table 2-8. Seven of the 15 studies examining serum testosterone levels in men have indicated associations between decreasing total and/or free testosterone levels and increasing urinary MEHP levels (Table 2-8). The association was seen in studies of men recruited from the general population (Chen et al. 2017; Joensen et al. 2012; Woodward et al. 2020) as well as among male partners of sub-fertile couples (Chang et al. 2017a, 2017b; Jurewicz et al. 2013; Meeker et al. 2009b; Wang et al. 2016). Of these studies, only Chang et al. (2017a, 2017b) observed an association between decreased testosterone and other DEHP metabolites (MEHHP, MEOHP, and MECPP). Woodward et al. (2020) reported decreasing total and free testosterone with increasing ΣDEHP metabolites in men ≥60 years old, but not younger men. One study (Chang et al. 2015) reported increased total and free testosterone with increasing serum MEHP metabolites in male partners of sub-fertile couples.

Table 2-8. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Occupationally exposed populations				
Fong et al. 2015 Occupational, 82 male PVC production workers (mean age 38 years), Taiwan	TT, E2, SHBG, LH, FSH, or Inhibin B	MEHP	IQR: 11.5–36.0 µg/g Cr	↔
		MEOHP	38.8–111.3	↔
Pan et al. 2006 Occupational, 74 exposed male PVC workers (mean age 33.5 years) and 63 unexposed male construction workers (mean age 34.3 years), China	**FT** E2, LH, or FSH	**MEHP**	Exposed: IQR: 209.6–1,884.4 µg/g Cr Unexposed: 3.7–9.9	↓
		MEHP	See above	↔
General population studies				
Axelsson et al. 2015 Cross-sectional, 314 men (age 17–20 years), Sweden	TT, FT, E2, SHBG, LH or FSH	MEHP	Range: 0.01–19 nmol/mmol Cr	↔
		MEHHP	0.5–340	↔
		MEOHP	0.2–200	↔
		MECPP	0.3–110	↔
Chang et al. 2019a, 2019b Cross-sectional, 207 elderly men (mean age 62.5 years) diagnosed with benign prostatic hyperplasia and prostatic enlargement, Taiwan	**E2**	**MEHP**	IQR: 1.42–5.50 ng/mL	↑
		MEHHP	2.99–13.1	↑
		MEOHP	2.39–8.95	↑
		MECPP	4.69–16.1	↑
	E1	MEHP, MEHHP, or MECPP	See above	↔
		MEOHP	See above	↑
	LH, FSH, SHBG, Inhibin B, TT, or FT	MEHP, MEHHP, MEOHP, or MECPP	See above	↔

Table 2-8. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Chen et al. 2017 Cross-sectional, 313 males age 12–30 years (and 473 females), Taiwan	**TT (male adults age ≥22–30 years)**	**MEHP**	Total (male and female, all ages) Mean (SD): 5.05 (12.86) µg/g Cr	↓
		MEHHP	26.70 (2.53)	↔
		MEOHP	16.65 (2.51)	↔
	TT (male adolescents age 12–<22 years)	MEHP, MEHHP, or MEOHP	See above	↔
Joensen et al. 2012	**TT, FT, or FSH**	**MEHP**	IQR: 0.4–18 ng/mL	↓
Cross-sectional, 881 men (age ~18–22 years), Denmark	E2, SHBG, LH, or Inhibin-B	MEHP	See above	↔
Jönsson et al. 2005 Cross-sectional, 234 men (age 18–21 years), Sweden	TT, E2, SHBG, LH, FSH, or Inhibin B	MEHP	IQR: <LOD–5.1 nmol/mmol Cr	↔
Meeker and Ferguson 2014 Cross-sectional, 867 males (age 12–80 years), United States (NHANES)	TT	ΣDEHP	NR	↔
		MEHP	12–20 years: IQR: 0.73–2.79 ng/dl 20–<40 years: 0.97–3.08 40–<60 years: 0.68–1.94 60–80 years: 0.58–2.09	↔
		MEHHP	12–20 years: 4.83–11.9 20–<40 years: 4.85–11.3 40–<60 years: 4.58–11.4 60–80 years: 5.08–11.8	↔
		MEOHP	12–20 years: 3.04–7.41 20–<40 years: 2.77–7.06 40–<60 years: 2.89–5.92 60–80 years: 3.42–7.92	↔

Table 2-8. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
		MECPP	12–20 years: 7.97–21.6 20–<40 years: 6.95–17.7 40–<60 years: 7.65–15.9 60–80 years: 8.45–19.7	↔
Woodward et al. 2020 Cross-sectional, 1,420 adult men (488 age 20–39 years, 457 age 40–59 years, 475 age ≥60 years), United States (NHANES)	**TT, E2, FT**	**ΣDEHP** (MEHP, MEHHP, MEOHP, MECPP)	All ages: IQR: 0.04–0.13 µmol/L	↔
			20–39 years: 0.04–0.12	↔
			40–59 years: 0.04–0.15	↔
			≥60 years: 0.04–0.13	↓
	SHBG	ΣDEHP	All ages and age groups: see above	↔
Populations recruited from fertility clinics				
Al-Saleh et al. 2019a Cross-sectional, 599 male partners (mean age 37.86 years) of infertile couples, Saudi Arabia	**FSH**	**ΣDEHP**	IQR: 0.161–0.433 µmol/L	↓
		MEHP	IQR: 9.467–22.368 µg/L	↔
		MEHHP	5.889–20.496	↓
		MEOHP	9.875–28.432	↓
		MECPP	17.044–53.328	↓
	Prolactin	**ΣDEHP or MEOHP**	See above	↓
		MEHP, MEHHP, or MECPP	See above	↔
	LH, E2, or TT	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	See above	↔
	WHO-diagnosed infertility in 47.7% of men, based on sperm concentration, motility, and morphology (≤15 million/mL, 32% and 4%, respectively).			

Table 2-8. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]		Result
Chang et al. 2015 Case-control, 176 men (age 25–45 years) including infertile men (n=141) and fertile men (n=35), Taiwan	**TT or FT**	ΣDEHP	Fertile Infertile 1 Infertile 2	GM (GSD): 0.11 (0.07) µmol/g Cr 0.12 (0.06) 0.14 (0.15)	↔
		MEHP	Fertile Infertile 1 Infertile 2	GM (GSD): 3.21 (0.30) µg/g Cr 4.11 (0.28) 4.52 (0.33)	↑
		MEHHP	Fertile Infertile 1 Infertile 2	8.30 (0.79) 9.94 (0.70) 10.1 (0.78)	↔
		MEOHP	Fertile Infertile 1 Infertile 2	6.14 (0.72) 5.85 (0.39) 5.66 (0.38)	↔
		MECPP	Fertile Infertile 1 Infertile 2	9.15 (1.01) 11.9 (0.83) 12.4 (0.85)	↔
	SHBG	ΣDEHP, MEHP, MEHHP, or MECPP	See above		↔
		MEOHP	See above		↑
	E2, LH, FSH, Inhibin B, or INSL3	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	See above		↔

Infertile men were classified as Infertile 1 (normal semen quality) and Infertile 2 (abnormal semen quality based on WHO reference values for semen volume and sperm concentration, motility and morphology).

Table 2-8. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Chang et al. 2017a, 2017b Cross-sectional, 253 male partners of sub-fertile couples from infertility clinics (124 men with normal semen quality, mean age 33.3 years; 129 men with abnormal semen quality, mean age 35.4 years) and 37 male partners of fertile couples (mean age 32.7 years), Taiwan	**TT**	**MEHP**	All men: IQR: 3.19–7.42 ng/mL	↓
		MEHHP	8.01–18.6	↓
		MEOHP	5.12–12.5	↓
		MECPP	10.1–23.4	↓
	E2	**MEHP, MEHHP, MEOHP, or MECPP**	See above	↑
	SHBG	MEHP	See above	↔
		MEHHP, MEOHP, or MECPP	See above	↑
	INSL3	**MEHP**	See above	↓
		MEHHP, MEOHP, or MECPP	See above	↔
	LH, FSH, or Inhibin B	MEHP, MEHHP, MEOHP, or MECPP	See above	↔
Jurewicz et al. 2013 Cross-sectional, 269 men (mean age 32 years) attending infertility clinic, Poland	**TT**	**MEHP**	Range: 0.5–399.3 µg/g Cr	↓
		MEOHP	1.2–131.0	↔
	E2 or FSH	MEHP or MEOHP	See above	↔

Table 2-8. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Mendiola et al. 2012 Cross-sectional, 850 men (425 male partners of pregnant women who conceived without assistance; 425 male partners of infertile couples), mean age 32.2 and 36 years, respectively, United States (California, Massachusetts, Minnesota, Missouri, New York, Iowa)	**FT**	**MEHP**	10^{th}–90^{th} percentile: 0.9–39.2 ng/mL	↓
		MEHHP	5.4–170	↓
		MEOHP	3.2–110	↓
	E2	**MEHP**	See above	↓
		MEHHP or MEOHP	See above	↔
	SHBG	MEHP	See above	↔
		MEHHP or MEOHP	See above	↑
	TT, LH, or FSH	MEHP, MEHHP, or MEOHP	See above	↔
	Among fertile men (Mendiola et al. 2011), a positive association was seen between MEHP and SHBG, but not for other metabolites or hormones. Among infertile men, negative associations were seen between SG-adjusted MEHP and total testosterone and estradiol levels (Meeker et al. 2009b).			
Pan et al. 2015 Cross-sectional,1,066 male partners of infertile couples (mean age 29.1 years), China	**E2 or INSL3**	**MEHP**	IQR: 2.4–8.7 ng/mL	↓
	TT, SHBG, LH, or FSH	MEHP	2.4–8.7	↔

Table 2-8. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Wang et al. 2016 Cross-sectional, 1,040 male partners of couples attending infertility clinic, China	**TT, FT, or E2**	**MEHP**	1st sample: IQR: 2.37–7.35 µg/g Cr 2nd sample: 2.53–8.80	↓
		MEHHP	1st sample: 6.80–15.07 2nd sample: 6.86–16.70	↔
		MEOHP	1st sample: 3.91–8.45 2nd sample: 3.94–9.27	↔
	FSH or LH	MEHP, MEHHP, or MEOHP	See above	↔

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; E1 = estrone (pg/mL); E2 = estradiol (pmol/L or pg/mL); FSH = follicle-stimulating hormone (IU/L); FT = free testosterone (nmol/L); GM = geometric mean; GSD = geometric standard deviation; INSL3 = insulin-like factor 3 (pg/mL); IQR = interquartile range; LH = luteinizing hormone (IU/L); LOD = limit of detection; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NHANES = National Health and Nutrition Examination Survey; NR = not reported; PVC = polyvinyl chloride; SD = standard deviation; SG-adj = specific gravity-adjusted; SHBG = sex hormone-binding globulin (nmol/mL or nmol/L); TT = total testosterone (nmol/L, ng/dL, ng/mL); WHO = World Health Organization

Among the remaining seven studies that did not observe any association with serum testosterone (Table 2-8), three studies (Al-Saleh et al. 2019a; Fong et al. 2015; Jönsson et al. 2005) did not report the timing of blood sample collection and did not consider time of sample collection in statistical analysis. Because serum testosterone levels vary over the course of the day, the lack of data on timing of sample collection (or consideration of timing in the statistical analysis) is an important limitation of these two studies. It is uncertain whether exposure levels differed among the positive and negative studies because the studies did not report urinary metabolite levels consistently. The available data do not indicate whether reductions were of a magnitude to be considered adverse, or whether the reductions were associated with other adverse effects.

Associations between urinary DEHP metabolites and estradiol levels in serum were also observed in males in several of these cross-sectional studies, although findings are inconsistent. Reduced serum estradiol was associated with increased urinary MEHP in four studies of male partners of sub-fertile couples (Meeker et al. 2009b; Mendiola et al. 2012; Pan et al. 2015; Wang et al. 2016), while another study observed increased serum estradiol with increasing urinary MEHP, MEHHP, MEOHP, and MECPP in sub-fertile men (Chang et al. 2017a, 2017b). In older men (≥60 years old), Woodward et al. (2020) reported decreased serum estradiol with increased ΣDEHP urinary metabolites in men. In contrast, Chang et al. (2019a) reported increased serum estradiol associated with increased urinary MEHP, MEHHP, MEOHP, and MECPP in older men (mean age 62.5 years) diagnosed with benign prostatic hyperplasia and prostatic enlargement. Increased serum estrone was also associated with increased urinary MEOHP in this study. No associations with urinary DEHP metabolites and serum estradiol were observed in the other cross-sectional studies of the general population (Axelsson et al. 2015; Joensen et al. 2012; Jönsson et al. 2005) or male partners of sub-fertile couples (Al-Saleh et al. 2019a; Chang et al. 2015; Jurewicz et al. 2013).

In cross-sectional studies of male partners of sub-fertile couples, four of five studies reported increased serum SHBG with increased urinary levels of MEHP (Mendiola et al. 2011), MEOHP (Chang et al. 2015, 2017a, 2017b; Mendiola et al. 2012), MEHHP (Chang et al. 2017a, 2017b; Mendiola et al. 2012), and/or MECPP (Chang et al. 2017a, 2017b); no association was observed with MEHP in the fifth study (no other metabolites were evaluated) (Pan et al. 2015). No association between DEHP exposure and serum SHBG was observed in the available general population studies (Chang et al. 2019a, 2019b; Joensen et al. 2012; Jönsson et al. 2005; Woodward et al. 2020).

Decreased serum FSH was associated with increased urinary metabolite levels in one study in male partners of sub-fertile couples (MEHHP, MEOHP, MECPP, or ΣDEHP) (Al-Saleh et al. 2019a) and one general population study (MEHP) (Joensen et al. 2012). This association was not observed in the remaining six studies in sub-fertile couples or three general population studies that examined serum FSH. None of the available studies observed a relationship between DEHP metabolites in urine and LH or inhibin B (Table 2-8).

Three of the cross-sectional studies examined serum levels of insulin-like factor 3 (INSL-3), a marker of Leydig cell function. Pan et al. (2015) and Chang et al. (2017a, 2017b) observed an inverse association between INSL-3 and urinary MEHP, while Chang et al. (2015) saw no relationship with any DEHP metabolite.

Two cohort studies and a number of cross-sectional studies have investigated relationships between urinary DEHP metabolite levels and semen parameters such as concentration, count, motility, and morphology. The studies selected for inclusion are in Table 2-9. Eleven of fifteen general population and fertility clinic patient studies did not show an association between sperm count and/or concentration and DEHP metabolites. Of the four showing associations, all were studies in males from sub-fertile couples. Two of these showed a negative association between sperm count/concentration and urinary DEHP metabolites (Chang et al. 2017a; Minguez-Alarcon et al. 2018a), while the other two showed a positive association (Al-Saleh et al. 2019a; Bloom et al. 2015a). Minguez-Alarcon et al. (2018a) also reported decreased percent normal sperm morphology with increasing MEHP levels; no association was observed with other urinary DEHP metabolites in this study. The other 12 studies evaluating sperm morphology did not observed an association with urinary DEHP metabolites.

Available studies evaluated sperm motility either as a continuous variable (n=10) or as a dichotomous variable (based on World Health Organization [WHO] reference values; n=5). When percent motile sperm was evaluated as a continuous variable, negative relationships were reported in five studies (Axelsson et al. 2015; Bloom et al. 2015a, 2015b; Chang et al. 2017a; Jurewicz et al. 2013; Mínguez-Alarcón et al. 2018a, 2018b), with another study reporting a positive relationship (Tian et al. 2019), and four other studies reporting no association (Al-Saleh et al. 2019a; Joensen et al. 2012; Jönsson et al. 2005; Pan et al. 2015). Studies that dichotomized percent motile sperm reported no association between risk of low motility sperm and DEHP urinary metabolites (Han et al. 2014b; Hauser et al. 2006; Herr et al. 2009; Liu et al. 2012; Wirth et al. 2008).

Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Sperm Parameters

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Occupationally exposed populations				
Huang et al. 2014a Occupational, 47 male PVC workers (36 with "high" exposure, mean age 36.3years; 11 with "low" exposure, mean age 35.5 years) and 15 unexposed men (mean age 25.3 years), Taiwan	**Sperm motility**	**MEHP**	High IQR: 11.5–31.9 µg/g Cr	↓
		MEHHP	47.1–111.5	↓
		MEOHP	41.0–99.4	↓
	Sperm concentration or morphology	MEHP, MEHHP, or MEOHP	See above	↔
General population studies				
Axelsson et al. 2015 Cross-sectional, 314 men (age 17–20 years), Sweden	**Sperm motility**	MEHP	Range: 0.01–19 nmol/mmol Cr	↔
		MEHHP	0.5–340	↓
		MEOHP	0.2–200	↓
		MECPP	0.3–110	↓
	Sperm count, concentration, or morphology	MEHP, MEHHP, MEOHP, or MECPP	See above	↔
Han et al. 2014b Cross-sectional, 232 adult men (age 20–40 years), China	Sperm count, concentration, morphology, or motility[b]	MEHP	5^{th}–95^{th} percentile: <LOD–31.4 µg/g Cr	↔
Joensen et al. 2012 Cross-sectional, 881 men (age ~18–22 years), Denmark	Sperm count, concentration, morphology, or motility	MEHP	IQR: 0.4–18 ng/mL	↔
Jönsson et al. 2005 Cross-sectional, 234 men (age 18–21 years), Sweden	Sperm count, concentration, or motility	MEHP	IQR: <LOD–12 nmol/mmol Cr	↔

Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Sperm Parameters

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Populations recruited from fertility clinics				
Al-Saleh et al. 2019a Cross-sectional, 599 male partners (mean age 37.86 years) of infertile couples, Saudi Arabia	**Sperm concentration**	**ΣDEHP**	IQR: 0.161–0.433 µmol/L	↑
		MEHP	IQR: 9.467–22.368 µg/L	↔
		MEHHP	5.889–20.496	↑
		MEOHP	9.875–28.432	↑
		MECPP	17.044–53.328	↑
	Sperm motility or morphology	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	See above	↔
	WHO-diagnosed infertility in 47.7% of men, based on sperm concentration, motility, and morphology (≤15 million/mL, 32%, and 4% respectively).			
Bloom et al. 2015a, 2015b Cohort, 473 male partners of infertile couples (mean age 31.8 years), United States (Michigan, Texas)	**Sperm motility**	MEHP	IQR: 0[c]–4.87 ng/mL	↔
		MEHHP	5.56–37.94	↔
		MEOHP	3.06–17.9	↓
		MECPP	8.60–46.4	↓
	Sperm count	MEHP, MEOHP, MECPP	See above	↔
		MEHHP	See above	↑
	Sperm morphology	MEHP, MEHHP, MEOHP, or MECPP	See above	↔
Chang et al. 2017a, 2017b Cross-sectional, 253 male partners of sub-fertile couples from infertility clinics (124 men with normal semen quality, mean age 33.3 years; 129 men with abnormal semen quality, mean age 35.4 years) and 37 male partners of fertile couples (mean age 32.7 years), Taiwan	**Sperm concentration or motility**	**MEHP**	All men: IQR: 3.19–7.42 ng/mL	↓
		MEHHP	8.01–18.6	↔
		MEOHP	5.12–12.5	↔
		MECPP	10.1–23.4	↔
	Sperm morphology	MEHP, MEHHP, MEOHP, or MECPP	See above	↔

Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Sperm Parameters

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Hauser et al. 2006 Cross-sectional, 463 male partners of sub-fertile couples (mean age 36.3 years), United States (Massachusetts)	Sperm concentration, morphology, or motility[b]	MEHP	IQR: 3.1–20.9 ng/mL (SG-adj)	↔
		MEHHP	23.4–113	↔
		MEOHP	15.8–73.0	↔
Herr et al. 2009 Cross-sectional, 349 male partners of sub-fertile couples (mean age 34.2 years), Germany	Sperm concentration, morphology, or motility[b]	ΣDEHP	IQR: 23.20–74.70 ng/mL	↔
Jurewicz et al. 2013 Cross-sectional, 269 men (mean age 32 years) attending infertility clinic, Poland	**Sperm motility**	**MEHP**	Range: 0.5–399.3 µg/g Cr	↓
		MEOHP	1.2–131.0	↓
	Sperm morphology or concentration	MEHP or MEOHP	See above	↔
Liu et al. 2012 Cross-sectional, 97 male partners of sub-fertile couples (mean age 31.5 years), China	Sperm motility or concentration[b]	MEHP	33rd–66th percentile: 0.35–1.93 µg/g Cr	↔
		MEOHP	1.89–3.05	↔
Mínguez-Alarcón et al. 2018a, 2018b Cohort, 936 male partners (median age 35.7 years) of couples seeking infertility treatment, United States (Massachusetts)	**Sperm concentration**	ΣDEHP	NR	↔
		MEHP	IQR: 1.12–7.00 ng/mL	↓
		MEHHP	6.44–32.9	↓
		MEOHP	3.82–20.1	↓
		MECPP	9.00–39.1	↔
	Sperm count	**ΣDEHP, MEHP, MEHHP, MEOHP, MECPP**	See above	↓
	Sperm motility or normal morphology	ΣDEHP, MEHHP, MEOHP, or MECPP	See above	↔
		MEHP	See above	↓

Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Sperm Parameters

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Pan et al. 2015 Cross-sectional,1,066 male partners of infertile couples (mean age 29.1 years), China	Sperm count, concentration, morphology or motility	MEHP	IQR: 2.4–8.7 ng/mL	↔
Tian et al. 2019 Cross-sectional, 86 men (mean age 31.6 years) undergoing fertility assessment, China	**Sperm motility**	**ΣDEHP**	IQR: 2.06–6.35 µg/g Cr	↑
		MEHP	0.25–2.77	↔
		MEOHP	1.48–3.76	↔
	Sperm count, concentration, or morphology	ΣDEHP, MEHP, or MEOHP	See above	↔
Wirth et al. 2008 Cross-sectional, 45 male partners of sub-fertile couples (mean age 34.8 years), United States (Michigan)	Sperm concentration, morphology or motility[b]	ΣDEHP	NR	↔
		MEHP	IQR: 4.6–22.1 ng/mL	NR
		MEHHP	32.7–137.1	NR
		MEOHP	20.1–79.3	NR

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.
[b]Analysis of sperm parameters was dichotomized based on WHO reference values for sperm concentrations (<20 million/mL), motility (<50% motile sperm), and/or morphology (<4% normal sperm)
[c]Value reported in study was less than zero, reflecting correction for analytical blank; adjusted to 0 for reporting in this table.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; IQR = interquartile range; LOD = limit of detection; max = maximum; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NR = not reported; PVC = polyvinyl chloride; SG-adj = specific gravity-adjusted; WHO = World Health Organization

The potential for paternal DEHP exposure to affect fertility or pregnancy outcome has not been well-studied. In prospective cohort studies, no associations were observed between paternal urinary DEHP metabolite levels and time-to-pregnancy (Buck Louis et al. 2014), *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) fertility rate (Al-Saleh et al. 2019b, 2019c), or preterm birth (Zhang et al. 2020a, 2020d). However, the probability of clinical pregnancy and live birth after IVF/ICSI was negatively associated with urinary paternal MEOHP and MECPP levels (Al-Saleh et al. 2019b, 2019c). A case-control study of 150 fertile and 139 infertile men showed that odds of infertility *decreased* with increasing urinary MEHP levels; no associations were observed for other DEHP metabolites (Liu et al. 2017). However, there is no information (qualitative or quantitative) on exposures prior to diagnosis; therefore, this study has limited usefulness for evaluating potential effects of DEHP exposure on male fertility.

One prospective cohort study of 68 sub-fertile men reported an association between increased preconception paternal urinary DEHP metabolite levels and decreased placental weight (Mustieles et al. 2019). While decreased placental weight may lead to intrauterine growth restriction (IUGR), low birth weight, or perinatal mortality, the findings are considered preliminary due to small sample size and inclusion of only sub-fertile men. No other studies of this endpoint were identified in the available literature.

Nonhuman Primate Studies—Male Reproductive Effects. Studies conducted in nonhuman primates generally indicate that they are not susceptible to DEHP-induced reproductive toxicity. A dose of 2,000 mg/kg/day given to 12–18-month-old marmoset monkeys for a 14-day period had no effect on testicular weight or histology (ICI Americas Inc. 1982; Rhodes et al. 1986). A 13-week gavage study in marmosets of unspecified age showed no significant treatment-related effects on gross or microscopic appearance of the testis or testicular zinc content at doses up to 2,500 mg DEHP/kg/day (Kurata et al. 1998).

Rodent Studies—Male Reproductive Effects. In the only available inhalation study evaluating male reproductive performance, no changes in fertility or mating performance of male Wistar rats were observed following exposure to DEHP during adulthood at concentrations up to 63 ppm for 6 hours/day, 5 days/week for 4 weeks (Klimisch et al. 1991, 1992). Mating with unexposed females was carried out at 2 and 6 weeks after the end of the DEHP exposure period. At sacrifice, there were no observable effects of DEHP on testicular structure.

Several studies evaluated reproductive performance in rats following oral exposure to DEHP. Two-generation studies in Wistar rats reported decreased F1 fertility after exposure to doses ≥1,040 mg/kg/day, but not ≤380 mg/kg/day (Schilling et al. 1999, 2001). It is likely that decreased fertility in F1 adults was due (at least in part) to male reproductive toxicity, because testes exhibited focal tubular atrophy at 113 mg/kg/day, and higher doses (≥1,040 mg/kg/day) resulted in aspermia, gross reproductive tract abnormalities, and decreased reproductive organ weights (Schilling et al. 1999, 2001). Testicular atrophy was also observed in F0 males at 1,088 mg/kg/day (Schilling et al. 2001).

Clear evidence of decreased male fertility in F1 and F2 generations was observed at doses ≥447 mg/kg/day in a 3-generation study in Sprague-Dawley rats via cross-over mating experiments; complete sterility was observed in F1 males at 659 mg/kg/day (Blystone et al. 2010; NTP 2005). Additional effects observed at doses ≥17 mg/kg/day included reproductive tract malformations in F1 and F2 adult offspring, and decreased reproductive organ weights, seminiferous tubule atrophy, epididymal aspermia, and decreased sperm counts in one or more generations. In 1-generation studies in which exposed male rats were mated to unexposed females following exposure for 21 days, decreased male fertility was only seen at ≥5,000 mg/kg/day (Dalgaard et al. 2000). This finding was accompanied by severe atrophy of seminiferous tubules, diffuse Leydig cell hyperplasia, and decreased testicular weights, with decreased seminal vesicle and epididymides weights occurring at 10,000 mg/kg/day (Dalgaard et al. 2000).

In a chronic exposure 2-generation study in Sherman rats, no changes in fertility or reproductive organ histology were observed; however, the highest dose evaluated was 200 mg/kg/day (Carpenter et al. 1953). Exposure to doses up to 1,156 mg/kg/day for 21–60 days prior to mating had no effect on male fertility (Agarwal et al. 1986; Dalgaard et al. 2000), even though male rats exposed to 1,156 mg/kg/day showed testicular atrophy, decreased sperm density and mobility, increased abnormal sperm, and decreased testes, epididymides, and prostate weights (Agarwal et al. 1986).

Reproductive performance has also been evaluated in mice following oral DEHP exposure. In a continuous breeding study, decreased fertility, and decreased numbers of litters/pair, pups/litter, live-born pups were observed at 130 mg/kg/day, with no litters produced at 390 mg/kg/day (Lamb et al. 1987; Morrissey et al. 1988; NTP 1984). Decreased fertility was attributed to both males and females in a cross-over mating trial, as fertility issues were observed when males exposed at 390 mg/kg/day were mated to unexposed females or vice versa. Additional reproductive effects observed in exposed males from the cross-over trial included decreased testes, epididymides, and prostate gland weights, decreased sperm concentration and motility, and increased percentages of abnormal sperm. In a 1-generation study

in mice, male and female exposure to doses up to 0.34 mg/kg/day for 2 weeks prior to mating through lactation did not result in any changes in mating, fertility, pregnancy outcomes, testes or epididymis weights, or sperm counts (Cha et al. 2018).

One study reported altered mating behavior in male C57Bl/6J mice 2 weeks after a 4-week exposure to DEHP at very low doses of ≥0.005 mg/kg/day, including increased latency to first intromission and ejaculation and reduced sexual interest in an unexposed female partner (Dombret et al. 2017). However, available data do not provide clear support for effects on male reproduction at these low doses. Specifically, mating indices were not affected in CD-1 mice exposed to doses up to 0.34 mg/kg/day for 2 weeks before mating and during mating (Cha et al. 2018). In addition, no effect on fertility was seen at doses up to 13 mg/kg/day in a continuous mating trial in Crl:CD-1 mice (Lamb et al. 1987; Morrissey et al. 1988; NTP 1984) or up to 180.77 mg/kg/day in a 2-generation study of CD-1 mice (Tanaka 2002). In rats, no effects on fertility were seen at doses up to 57 mg/kg/day in a 3-generation continuous mating study (Blystone et al. 2010; NTP 2005) or at doses up to 380 mg/kg/day in 2-generation studies (Schilling et al. 1999, 2001). Additionally, male mating behavior in rats was not affected by developmental exposure to DEHP at doses up to 100 mg/kg/day from GD 1 to PND 21 (Dalsenter et al. 2006) or 405 mg/kg/day from GD 6 to PND 21 (Andrade et al. 2006a); see Section 2.17 for more details. Based on the lack of effects on reproduction at doses much higher than those used by Dombret et al. (2017), the effects in this study are considered to be of uncertain toxicological significance; therefore, the study is not included in the LSE table.

Additional studies that did not evaluate reproductive performance indicate that the testes are a primary target tissue of DEHP toxicity in adult rats. In an acute study, moderate to severe changes in seminiferous tubules and decreased testes weight were observed at doses ≥1,000 mg/kg/day (Dostal et al. 1988). In a 9-week study, the percentage of sperm with bent tails was increased at ≥0.1 mg/kg/day and the percentage of normal sperm was decreased at 1 mg/kg/day (Hsu et al. 2016). No changes in sperm count or motility or reproductive organ weights were observed; reproductive organ histology was not assessed. At 1 mg/kg/day, there was a decreased normal sperm percentage, sperm DNA fragmentation, and increased hydrogen peroxide production from sperm. DNA fragmentation was associated with increased hydrogen peroxide production (Hsu et al. 2016). In other intermediate-duration studies, the lowest doses associated with mild to moderate testicular lesions were 37.6 mg/kg/day (Poon et al. 1997) and 142 mg/kg/day (Gray et al. 1977; lowest dose tested).

Additional effects in intermediate-duration studies, including testicular atrophy and degeneration, degeneration of the Leydig cells, decreased spermatogenesis/hypospermia, interstitial edema, and decreased testicular weights, were observed at ≥300 mg/kg/day in F344 rats (CMA 1986; Exxon Chemical Americas 1990; Myers 1992b; NTP 1982), Wistar rats (Shaffer et al. 1945), and Sprague-Dawley rats (Wang et al. 2020). However, three intermediate-duration studies reported no histopathological changes in the testes at doses up to 200 mg/kg/day for 28 days in Long-Evans rats (Akingbemi et al. 2001), 930 mg/kg/day for 3 weeks in F344 rats (Astill et al. 1986), or 3,000 mg/kg/day for 30 days in Wistar rats (Wang et al. 2020). In chronic studies, the lowest doses associated with testicular effects (spermatogenesis and seminiferous tubule degeneration) were 14 and 29 mg/kg/day (David et al. 2000a; Ganning et al. 1991). Severe degeneration, atrophy, and decreased testes weights were reported at chronic doses ≥300 mg/kg/day (David et al. 2000a; Kluwe et al. 1982a; Price et al. 1987; Voss et al. 2005).

Similarly, studies that did not evaluate reproductive performance also clearly indicate that the testes are a primary target tissue of DEHP toxicity in adult mice, although the effective dose appears to differ among strains. The most sensitive strain is A/J mice, with Sertoli cell vacuolation and germ cell sloughing in seminiferous tubules after exposure to dietary doses ≥12.3 mg/kg/day for 2–8 weeks (Kitaoka et al. 2013). Lymphocyte infiltration in the testes and hypospermia in the seminiferous tubules were also observed at ≥12.3 and 125 mg/kg/day, respectively, after 8 weeks (Kitaoka et al. 2013). In ICR mice, decreased testes weights were observed following a 3-week exposure to 180 mg/kg/day (Feng et al. 2020); however, increased testes weights were observed following a 4-week exposure to 400 mg/kg/day (Feng et al. 2020; Xu et al. 2019).

No histopathological or spermatogenesis changes were observed at doses up to 400 mg/kg/day for 4 weeks (Xu et al. 2019). In BALB/c mice, slight localized degeneration of germ cells was observed after a 30-day exposure to ≥1,000 mg/kg/day (Wang et al. 2020). However, in B6C3F1 mice, testicular effects (testicular atrophy, decreases/absent spermatogenesis, and decreased testes/epididymides weights) were observed after intermediate-duration exposure to doses ≥2,579 mg/kg/day, but not ≤2,500 mg/kg/day (Myers 1992a; NTP 1982). Similarly, slight seminiferous tubule atrophy was observed in C57BL/6J mice after a 30-day exposure to 3,000 mg/kg/day, but not ≤1,000 mg/kg/day (Wang et al. 2020). In C57Bl/6J × BALBcByJ hybrid mice, exposure to 1,100 mg/kg/day (only dose tested) for 26 weeks resulted in decreased testes weights and focal testicular atrophy (Toyosawa et al. 2001). Chronic exposure of B6C3F1 mice resulted in bilateral hypospermia, immature/abnormal sperm in the epididymides, and

decreased testes weights at doses ≥292 mg/kg/day and seminiferous tubule degeneration at 1,325 mg/kg/day (David et al. 2000b; Kluwe et al. 1982a; NTP 1982).

There is some evidence for altered male reproductive hormones in adult rodents exposed to high levels of DEHP. While no changes in serum testosterone or LH were observed in adult Long-Evans rats following exposure to doses up to 750 mg/kg/day for 14 days, exposure for 21–35 days resulted in decreased serum testosterone and increased serum LH at doses ≥10 mg/kg/day (Li et al. 2012a). No changes in serum testosterone or LH levels were observed in adult Long-Evans rats at doses up to 200 mg/kg/day for 28 days (Akingbemi et al. 2001). In 30-day drinking water studies, significant decreases in serum testosterone were observed in Sprague-Dawley rats at 3,000 mg/kg/day (60% decrease) and BALB/c mice at ≥1,000 mg/kg/day (33–39% decrease) (Wang et al. 2020). No exposure-related changes were observed in similarly exposed Wistar rats or C57BL/6J mice at doses up to 3,000 mg/kg/day. In ICR mice, no exposure-related changes were observed in serum testosterone following a 28-day exposure to doses up to 400 mg/kg/day (Xu et al. 2019).

It is not clear whether DEHP has antiandrogenic potential when using the Hershberger assay. In the Hershberger assay, male rats were castrated and subsequently supplemented with testosterone so control and exposed animals had equivalent testosterone levels. In Sprague-Dawley rats exposed to DEHP for 10 days, Lee and Koo (2007) observed significantly decreased ventral prostate weights at ≥20 mg/kg/day (lowest dose tested), decreased seminal vesicle weights and increased serum LH at ≥100 mg/kg/day, and decreased levator ani/bulbocavernosus (LABC) muscle weights at 500 mg/kg/day. There were no exposure-related changes in serum testosterone. Using the same rat strain, duration of exposure, and number of rats per group, Kim et al. (2018b) did not observe any dose-related changes in ventral prostate, seminal vesicle, coagulating glands, LABC muscle, paired Cowper's glands, or glans penis following exposure to doses up to 400 mg/kg/day with or without testosterone supplementation; reproductive hormones were not assessed. However, using Wistar rats, Stroheker et al. (2005) observed decreased prostate weights at ≥200 mg/kg/day, decreased seminal vesicle weights at ≥400 mg/kg/day, and significantly decreased LABC muscle weights at ≥100 mg/kg/day; but had no findings at ≤20 mg/kg/day. As expected, no exposure-related changes in serum testosterone were observed. Reproductive organ histology was not assessed in any of the Hershberger assays.

In intact (not castrated) rats, no changes in prostate weight were at doses up to 150 mg/kg/day for 13 weeks starting on PND 6 (Kim et al. 2018c). Reproductive hormones and organ histology were not assessed by Kim et al. (2018c). Another study with intact mice reported a significant 16% increase in

absolute prostate weight (without a change in body weight) after a 28-day exposure to 400 mg/kg/day (Xu et al. 2019). No histopathological changes were observed for the prostate in this study (Xu et al. 2019).

Observed alterations in hormone levels may be due to Leydig cell toxicity. In Long-Evans rats, exposure to DEHP at doses ≥10 mg/kg/day for 7–11 days resulted in an increase in the number of Leydig cells in the testes (Guo et al. 2013). When mature Leydig cells were eliminated using ethane dimethane sulfonate (EDS), a significant increase in the number and proliferation of Leydig cell precursors was observed following exposure to ≥10 mg/kg/day for 11–35 days (Guo et al. 2013; Li et al. 2012a). However, no changes were observed in Leydig cell testosterone production *in vivo* in cells harvested from adult Long-Evans rats exposed to doses up to 200 mg/kg/day for 28 days (Akingbemi et al. 2001).

Other Mammalian Species—Male Reproductive Effects. In ferrets, absence of germinal epithelium in the seminiferous tubules was observed in 3/7 animals exposed to 1,200 mg/kg/day for 14 months (only dose tested) (Lake et al. 1976). Relative testes weights were also elevated at this dose, but this effect appeared to be secondary to exposure-related weight loss.

Mechanisms of Male Reproductive Toxicity. As discussed above, several studies suggest associations between diminished semen quality and DEHP metabolite levels in urine. Additionally, Zhang et al. (2006) reported an association between increased DEHP metabolite levels in semen and altered semen parameters (decreased semen volume, increased rate of sperm malformation). Some studies have indicated that oxidative stress may potentially be a mechanism of toxicity for observed alterations in male semen quality (Hoyer et al. 2018; Shen et al. 2018). In a study in PVC workers, increased urinary DEHP metabolite levels were associated with both decreased sperm motility and sperm ROS generation (Huang et al. 2014a).

Studies reported associations between urinary DEHP metabolite levels and urinary markers of oxidative stress (e.g., 8-hydroxy-2'-deoxyguanosine [8-OHdG], isoprostane, carnitines) in couples planning to become pregnant (Guo et al. 2014), couples seeking fertility treatment (Wu et al. 2017), and men from a fertility cohort (Zhang et al. 2016); however, these studies do not have concurrent evaluations of male reproductive parameters. Direct damage to sperm DNA may also underlie observed male reproductive effects, as increased urinary levels of DEHP metabolites were associated with DNA damage in men from a fertility cohort (Hauser et al. 2007). *In vitro* studies using human sperm suggest that mechanisms of altered sperm function induced by DEHP and MEHP may include DNA fragmentation or altered calcium signaling (Sumner et al. 2019; Sun et al. 2020).

Decreased testosterone production was observed in adult human testes explants cultured with DEHP or MEHP (Desdoits-Lethimonier et al. 2012). No effects were observed on INSL3 production by Leydig cells, inhibin B production by Sertoli cells, or germ cell apoptosis, suggesting that effects were limited to steroidogenesis. DEHP can alter sterologenesis in the liver of rodents, which may have an impact on steroid-dependent functions. For example, feeding male rats DEHP at an estimated dose of 500 mg/kg/day for 7–18 days significantly inhibited sterologenesis from ^{14}C-mevalonate in liver and adrenal minces (Bell 1976, 1980). Other mechanisms may include apoptosis, as germ cell apoptosis was observed following gavage administration of MEHP to prepubertal rats and mice (Lagos-Cabre and Moreno 2012). Germ cell apoptosis appears to be mediated by upregulation of FasL (an apoptosis-related protein in Sertoli cells) (Lagos-Cabre and Moreno 2012).

Mechanisms of male reproductive toxicity occurring after gestational or early postnatal exposure to DEHP are in Section 2.17 (Developmental; Mechanisms of Altered Male Reproductive Development).

Epidemiology Studies —Female Reproductive Effects. Few epidemiological studies evaluating the effects of exposure to DEHP on the female reproductive system met inclusion criteria (Appendix B). Many of the available studies (Barrett et al. 2014; Buck Louis et al. 2013; Grindler et al. 2015; Huang et al. 2010, 2014a; Itoh et al. 2009; Kim et al. 2015; Lee et al. 2020; Pollack et al. 2015; Sun et al. 2016; Upson et al. 2013; Weuve et al. 2010; Velez et al. 2015) measured exposure using urine samples collected after the outcome of interest (e.g., pregnancy, endometriosis, fibroids, early menopause, etc.) had occurred, limiting their utility for assessing the potential cause and effect relationship. Others were excluded because exposure was assessed using biomarkers other than urinary metabolites (Caserta et al. 2013; Cobellis et al. 2003; Du et al. 2016; Kim et al. 2011; La Rocca et al. 2014; Reddy et al. 2006; Romani et al. 2014; Specht et al. 2015). Studies that met inclusion criteria are presented in Table 2-10 and discussed below.

Three prospective cohort studies of couples discontinuing birth control to become pregnant did not observe associations between DEHP exposure and prolonged time to pregnancy (Buck Louis et al. 2014; Jukic et al. 2016; Thomsen et al. 2017). One of these studies (Jukic et al. 2016) evaluated the menstrual cycle, observing that most DEHP metabolites were not associated with altered luteal or follicular phase length. Out of three prospective cohort studies of females seeking IVF or ICSI treatment (Al-Saleh et al. 2019b, 2019c, 2019d; Deng et al. 2020; Machtinger et al. 2018), one reported decreased fertilization rate with increased maternal DEHP urinary metabolites (Machtinger et al. 2018). Two cohort studies in IVF

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Female Reproductive Effects

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Women recruited when trying to get pregnant or from infertility clinics				
Al-Saleh et al. 2019b, 2019c, 2019d Cohort, 599 female partners (mean age 32.8 years) of couples seeking IVF/ICSI treatment, Saudi Arabia	Fertilization rate	ΣDEHP	IQR: 0.137–0.381 µmol/L	↔
		MEHP	IQR: 8.73–21.7 µg/L	↔
		MEHHP	4.68–16.4	↔
		MEOHP	9.37–31	↔
		MECPP	14.2–44.9	↔
Buck Louis et al. 2014 Cohort, 454 women (age 18–44 years), recruited when attempting to become pregnant, United States (Michigan, Texas)	Fecundability	MEHP	Pregnant: IQR: 4.56 (3.40–6.11) ng/mL Not pregnant: 5.60 (3.81–8.24)	↔
		MEHHP	Pregnant: 15.24 (13.01–17.86) Not pregnant: 14.46 (11.52–18.14)	↔
		MEOHP	Pregnant: 8.65 (7.40–10.10) Not pregnant: 7.55 (5.86–9.74)	↔
		MECPP	Pregnant: 21.18 (18.25–24.58) Not pregnant: 21.21 (16.94–26.55)	↔
Deng et al. 2020 Cohort, 663 women receiving IVF/ICSI treatment (mean age 31.3 years), China	Fertilization, number of retrieved and mature oocytes, good quality embryos (day 3), or total and good quality blastocyte formation	MEHP	IQR: 3.85–16.77 µg/g Cr	↔
		MEHHP	7.47–18.87	↔
		MEOHP	4.94–13.59	↔
Hauser et al. 2016 Cohort, 256 women (age 21–43 years) undergoing IVF, United States (Massachusetts)	**Number of total and mature oocytes**	**ΣDEHP**	IQR: 0.10–0.42 µmol/L (SG-adj)	↓
		MEHP	IQR: 1.37–6.87 µg/L (SG-adj)	↓
		MEHHP	7.75–35.0	↓
		MEOHP	5.48–25.4	↓
		MECPP	14.6–57.2	↓

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Female Reproductive Effects

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Jukic et al. 2016 Cohort, 221 women (median age 29 years), recruited when attempting to become pregnant, United States (North Carolina)	**Luteal phase length**	ΣDEHP	NR	↔
		MEHP	IQR: 3.8–11.2 ng/mL	↔
		MEHHP	31.8– 80.8	↔
		MEOHP	19.5–48.9	↔
		MECPP	42.2–100.0	↑
	Fecundability or Follicular phase length	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	See above	↔
Machtinger et al. 2018 Cohort, 136 women (mean age 30.9 years) receiving IVF treatment, Israel	**Number of total and mature oocytes or top-quality embryos**	**ΣDEHP**	IQR: 0.11–0.27 µmol/L (SG-adj)	↓
		MEHP	IQR: 2.2–7.6 µg/L (SG-adj)	↔
		MEHHP	8.6–22.2	↓
		MEOHP	6.4–16.1	↓
		MECPP	13.3–33.6	↓
	Number of fertilized oocytes	**ΣDEHP, MEHHP, or MEOHP**	See above	↓
		MEHP, MECPP	See above	↔
	Probability of implantation	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	See above	↔
Messerlian et al. 2016a Cohort, 215 women (age 20–45 years) seeking infertility investigation, United States (Massachusetts)	**Antral follicle count**	**ΣDEHP**	IQR: 0.10–0.46 µmol/L (SG-adj)	↓
		MEHP	IQR: 1.6–6.7 µg/L (SG-adj)	↓
		MEHHP	8.2–41.1	↔
		MEOHP	5.1–25.0	↓
		MECPP	13.5–59.1	↓

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Female Reproductive Effects

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Thomsen et al. 2017 Cohort, 229 women (age 20–35 years), recruited when attempting to become pregnant, Denmark	Fecundability	MEHP	Median (range): 14.5 (0–348) ng/mL	↔
Pregnant women				
Johns et al. 2015 Cross-sectional, 106 pregnant women (age 18–40 years), Puerto Rico	Estradiol, SHBG, or progesterone	ΣDEHP	NR	↔
		MEHP	GWs 16–20: IQR: 1.61–6.36 ng/mL (SG-adj) GWs 24–28: 1.69–6.73	NR
		MEHHP	GWs 16–20: 6.14–19.9 GWs 24–28: 7.28–16.9	NR
		MEOHP	GWs 16–20: 5.57–16.5 GWs 24–28: 6.22–14.8	NR
		MECPP	GWs 16–20: 12.7–31.4 GWs 24–28: 13.4–29.3	NR
Sathyanarayana et al. 2017 Cross-sectional, 591 pregnant women (age 20–40 years), United States (California, Minnesota, New York, Washington)	**Estrone or estradiol**	ΣDEHP	IQR: 15.73–39.70 ng/mL (SG-adj)	↔
		MEHP	1.38–4.35	↑
		MEHHP	4.35–12.66	↔
		MEOHP	3.22–8.46	↑
		MECPP	5.89–15.71	↔
	Total testosterone	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	See above	↔
	Free testosterone	ΣDEHP, MEHP, MEHHP, or MEOHP	See above	↔
		MECPP	See above	↓

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Female Reproductive Effects

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Sathyanarayana et al. 2014 Cross-sectional, 180 pregnant women (age 20–40 years; 94 with male fetuses, 86 with female fetuses), United States (California, Minnesota, Missouri)	**Total or free testosterone**	**ΣDEHP** (MEHP, MEHHP, MEOHP)	IQR: 5.53–21.05 µmol/L	M fetus: ↔ **F fetus: ↓**
	Estradiol	ΣDEHP	See above	M, F fetus: ↔
Nonpregnant women (general population)				
Meeker and Ferguson 2014 Cross-sectional, 697 women (age 20–80 years), United States (NHANES)	Testosterone	ΣDEHP	NR	↔
		MEHP	20–<40 years: IQR:1.07–3.57 ng/mL (Cr-adj) 40–<60 years: 0.90–2.90 60–80 years: 0.70–1.94	↔
		MEHHP	20–<40 years: 5.44–14.6 40–<60 years: 5.44–14.6 60–80 years: 5.27–13.7	↔
		MEOHP	20–<40 years: 3.62–10.0 40–<60 years: 3.73–10.0 60–80 years: 3.41–8.38	↔
		MECPP	20–<40 years: 9.06–21.7 40–<60 years: 10.4–23.9 60–80 years: 9.98–23.9	↔

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; Cr = creatinine; Cr-adj = creatinine-adjusted; DEHP = di(2-ethylhexyl)phthalate; GW = gestation week; ICSI = intracytoplasmic sperm injection; IQR = interquartile range; IVF = *in vitro* fertilization; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NHANES = National Health and Nutrition Examination Survey; NR = not reported; SHBG = sex hormone binding globulin; SG-adj = specific gravity-adjusted

patients also reported decreased number of total and mature oocyte and/or decreased top-quality embryos with increased maternal DEHP urinary metabolites (Hauser et al. 2016; Machtinger et al. 2018). Another cohort study of women seeking evaluation for fertility problems observed decreases in ovarian antral follicle counts (AFCs) associated with higher DEHP metabolite concentrations in urine samples collected before AFCs were determined (Messerlian et al. 2016a). Multiple urine samples were collected for some of the women in this study, improving exposure estimates; however, the small population size and lack of evidence for decreased fertility in prospective cohort studies make the findings inconclusive.

Four cross-sectional studies evaluating whether DEHP exposure alters reproductive hormones in women are limited and reported inconsistent findings (Table 2-10). A cross-sectional study in 591 pregnant women reported increased serum estrone and estradiol with increased MEHP and MEOHP urinary levels; no associations were observed with the sum of DEHP metabolites (Sathyanarayana et al. 2017). Two additional cross-sectional studies (n≤180) did not report an association between serum estradiol and urinary DEHP metabolites in pregnant women (Johns et al. 2015; Sathyanarayana et al. 2014). In addition, Johns et al. (2015) observed no association with serum SHBG or progesterone. Reduced free testosterone in pregnant women was associated with higher urinary MECPP levels, but not levels of other DEHP metabolites, and no associations were observed between DEHP metabolites and total testosterone (Sathyanarayana et al. 2017). Sathyanarayana et al. (2014) observed associations between reduced total and free serum testosterone and higher urinary metabolite concentrations in women delivering female infants, but no association in women delivering male infants. In a cross-sectional study of women between 20 and 80 years of age who participated in the 2011–2012 NHANES survey, while urinary metabolite levels were generally associated with lower serum total testosterone, no association was seen for any DEHP metabolite or age group (Meeker and Ferguson 2014).

Epidemiology Studies—Pregnancy Outcomes. Several cohort and case-control studies have evaluated potential associations between pregnancy outcomes (e.g., gestational age, pre- or post-term birth, pregnancy loss; Table 2-11).

Preterm birth as a categorical measure (<37 weeks of gestation) was evaluated in 10 epidemiological studies. Six of these studies reported increased odds of preterm birth associated with increased urinary DEHP metabolites, including cohort studies (Bloom et al. 2019a; Ferguson et al. 2019a, 2019b; Gao et al. 2019; Zhang et al. 2020a, 2020d) and case-control studies (Ferguson et al. 2014b, 2014c; Meeker et al. 2009a). In some of these studies, increased odds were only observed in a subset of study subjects. For example, Bloom et al. (2019a) found an association between preterm birth and urinary MEHP only in

Table 2-11. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Adibi et al. 2009 Cohort, 283 pregnant women (mean age 30.2 years), United States (California, Iowa, Minnesota, Mississippi)	**Preterm birth (<37 weeks)**	**MEHP**	IQR: 1.1–8.2 ng/mL	↓
		MEHHP	5.6–25.5	↓
		MEOHP	5.1–24.6	↓
	Post-term birth (>41 weeks) or Gestational age	**MEHP, MEHHP, MEOHP**	See above	↑
Al-Saleh et al. 2019d Cohort, 599 women seeking IVF/ICSI treatment (mean age 32.8 years), Saudi Arabia	**Failed biochemical pregnancy (early pregnancy loss), failed clinical pregnancy or failed live birth**	ΣDEHP	IQR: 0.137–0.381 µmol/L	↔
		MEHP	IQR: 8.73–21.7 µg/L	↑
		MEHHP	4.68–16.4	↔
		MEOHP	9.37–31	↔
		MECPP	14.2–44.9	↔
		Al-Saleh et al. (2019c, 2019d) did not report increased risk of biochemical pregnancy and failed live birth with increased urinary MEHP in this cohort; however, models were not adjusted for male partner urinary phthalate levels.		
Bloom et al. 2019a, 2019b Cohort, 310 mother-infant pairs (152 African American and 158 White mothers; mean age 27.6 years), urinary metabolites measured at 18–22 weeks (1st visit) and 24–32 weeks (2nd visit); United States (South Carolina)	**Preterm birth**	ΣDEHP	All women (1st visit): IQR: 33.5–92.0 nmol/L (SG-adj)	↔
			All women (2nd visit): 37.8–81.7	↔
			African American (1st visit): 21.5–69.4	↔
			White (1st visit): 22.1–52.1	↔
		MEHP	All women (1st visit): IQR: 1.5–5.3 ng/mL (SG-adj)	↔
			All women (2nd visit): 1.4–4.5	↔
			African American (1st visit): 1.0–4.1	↔
			White (1st visit): 0.8–2.6	↑
		MEHHP	All women (1st visit): 3.5–9.1	↔
			All women (2nd visit): 3.5–8.1	↔
			African American (1st visit): 2.5–7.9	↔
			White (1st visit): 2.8–6.1	↔

Table 2-11. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
		MEOHP	All women (1st visit): 4.1–12.2	↔
			All women (2nd visit): 4.7–10.9	↔
			African American (1st visit): 2.1–5.8	↔
			White (1st visit): 2.1–5.0	↔
		IQRs were estimated from graphically presented data using GrabIt! software.		
Casas et al. 2016 Cohort, 657 pregnant women (age ≥16 years), Spain	Gestational age	ΣDEHP	Range: 26.5–1,670 µg/g Cr	↔
Deng et al. 2020 Cohort, 663 women receiving IVF/ICSI treatment (mean age 31.3 years), China	Rate of clinical pregnancy, live birth, or early miscarriage	MEHP	IQR: 3.85–16.77 µg/g Cr	↔
		MEHHP	7.47–18.87	↔
		MEOHP	4.94–13.59	↔
Ferguson et al. 2014b Case-control,130 preterm births (<37 weeks; median age 32.8 years) and 352 random controls (≥37 weeks; median age 32.7 years), United States (Massachusetts)	**Preterm birth**	**ΣDEHP**	IQR: 20.2–63.2 µmol/mL (SG-adj)	↑
		MEHP	5.51–18.1	↑
		MEHHP	17.2–55.3	↔
		MEOHP	9.33–29.7	↔
		MECPP	20.6–73.8	↑
	Spontaneous preterm birth	**ΣDEHP, MEHP, MEOHP, or MECPP**	See above	↑
		MEHHP	See above	↔
		Using the same study population, Ferguson et al. (2014c) evaluated associations of DEHP metabolites at four timepoints during pregnancy with preterm birth. Positive associations were observed between spontaneous preterm birth and MECPP and ΣDEHP at visit 3 (22.9–29.3 weeks of gestation).		

Table 2-11. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Ferguson et al. 2019c Cohort, 1,090 pregnant women examined at median GWs 17.6 (visit 1), 23.4 (visit 2), and 27.6 (visit 3) (age ≥18–<40 years), Puerto Rico	Gestational age, Preterm birth, or Spontaneous preterm birth	ΣDEHP	NR	↔
		MEHP	GM (visits 1–3): 2.30 ng/mL (SG-adj)	NR
		MEHHP	7.31	NR
		MEOHP	6.38	NR
		MECPP	13.3	NR
Ferguson et al. 2019a, 2019b Cohort, 783 pregnant women (age ≥18 years), including 281 women with at least 1 stressful life event (SLE) during pregnancy and 429 with no SLE during pregnancy, United States (California, New York, Minnesota, Washington)	**Preterm birth or Spontaneous preterm birth (all women)**	**ΣDEHP**	Pregnancy average: NR	
			1st trimester: IQR: 0.05–0.14 nmol/L (SG-adj)	↔
			2nd trimester: 0.05–0.14	↔
			3rd trimester: 0.06–0.14	↑
	Preterm birth (women with SLE)	**ΣDEHP**	3rd trimester: 0.05–0.14	↑
	Preterm birth (women without SLE)	ΣDEHP	3rd trimester: 0.06–0.14	↔
	SLE included job loss, serious illness, family death, relationship difficulties with spouse/partner, and legal or financial problems.			
Gao et al. 2017 Cohort, 3,103 mother-infant pairs (mean age 26.4 years), China	Gestational age	ΣDEHP	NR	↔
		MEHP	25th–95th percentile: 1.34–13.86 µg/g Cr	NR
		MEHHP	3.01–20.19	NR
		MEOHP	4.32–23.05	NR

Table 2-11. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Gao et al. 2019 Cohort, 3,266 pregnant women, 19 very preterm (<33 weeks), 115 late preterm (34–36 weeks), 791 early-term (37–38 weeks), 1,986 full-term (39–40 weeks), 344 late-term (41 weeks), and 11 post-term (>42 weeks) births (mean age 26.61 years), China	**Preterm birth (overall <37 weeks) or post-term birth**	ΣDEHP	NR	↔
		MEHP	IQR: 3.45–9.61 µg/g Cr	↑
		MEHHP	5.62–14.16	↔
		MEOHP	7.15–15.42	↔
	Gestational age, Very preterm birth, late preterm birth, early-term birth, or late-term birth	ΣDEHP, MEHP, MEHHP, or MEOHP	See above	↔
Hu et al. 2020 Cohort, 1,857 pregnant women (age ≥19 years), Canada	Preterm birth (<37 weeks), spontaneous preterm birth, or gestational age	ΣDEHP	Maternal (1st trimester): IQR: 0.04–0.11 µmol/L (SG-adj)	↔
		MEHP	IQR: 1.5–4.1 µg/L (SG-adj)	↔
		MEHHP	6.4–16.3	↔
		MEOHP	4.6–11.1	↔
Jukic et al. 2016 Cohort, 221 healthy women (median age 26 years), United States (North Carolina)	**Early pregnancy loss**	**ΣDEHP**	NR	↓
		MEHP	IQR: 3.8–11.2 ng/mL	↔
		MEHHP	31.8–80.8	↔
		MEOHP	19.5–48.9	↓
		MECPP	42.2–100.0	↔
Machtinger et al. 2018 Cohort, 136 women (mean age 30.9 years) receiving IVF treatment, Israel	Clinical pregnancy or Live birth	ΣDEHP	IQR: 0.11–0.27 µmol/L (SG-adj)	↔
		MEHP	IQR: 2.2–7.6 µg/L (SG-adj)	↔
		MEHHP	8.6–22.2	↔
		MEOHP	6.4–16.1	↔
		MECPP	13.3–33.6	↔
	Preterm birth	**ΣDEHP**	Controls: IQR: 0.16–0.55 µg/g Cr Cases: 0.28–0.45	↑
		MEHP	Controls: 1.7–7.4 Cases: 3.3–7.4	↔

Table 2-11. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Meeker et al. 2009a Case-control, 30 preterm births and 30 controls (median age 27 years), Mexico		MEHHP	Controls: 11.4–52.1 Cases: 24.1–41.5	↔
		MEOHP	Controls: 9.5–42.1 Cases: 20.6–29.2	↔
		MECPP	Controls: 27.3–98.6 Cases: 52.7–77.4	↔
Messerlian et al. 2016b Cohort, 256 women (with 303 conceived pregnancies) undergoing medically assisted reproduction (mean age 34.9 years), United States (Massachusetts)	**Early pregnancy loss**	ΣDEHP	IQR: 0.10–0.40 µmol/L	↔
		MEHP	IQR: 1.5–6.4 ng/mL	↔
		MEHHP	7.8–35.4	↔
		MEOHP	5.5–24.4	↑
		MECPP	14.3–57.2	↔
	Pregnancy loss (total)	ΣDEHP, MEHP, or MECPP	See above	↔
		MEHHP or MEOHP	See above	↑
Mu et al. 2015a Case-control, 132 cases of spontaneous abortion and 172 controls (age 20–45 years), China	Pregnancy loss (clinical)	MEHP	Cases: 5^{th}–95^{th} percentiles: 1.53–103 µg/g Cr Controls: 1.27–20.8	↔
Shoaff et al. 2016 Cohort, 368 mother-infant pairs (age ≥18 years), United States (Ohio)	Preterm birth or gestational age	ΣDEHP	16 weeks: IQR: 0.14–0.72 nmol/mL 26 weeks: 0.10–0.52 nmol/mL	↔
Su et al. 2014 Cohort,130 mother-infant pairs (maternal age NR), Taiwan	Gestational age	ΣDEHP	95% CI: 42.28–60.83 µg/g Cr	↔
		MEHP	14.56–20.19	↔
		MEHHP	5.49–10.53	↔
		MEOHP	10.05–17.58	↔

Table 2-11. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Toft et al. 2012 Cohort, 128 pregnant women including 48 with pregnancy loss and 80 with a liveborn child (age 20–35 years), Denmark	**Early pregnancy loss**	**MEHP**	Pregnancy loss: range: <LOD–84 µg/L Liveborn child: <LOD–64	↑
		MEHHP	Pregnancy loss: 9.5–207.1 Liveborn child: 3.6–215.3	↔
		MEOHP	Pregnancy loss: 5.7–245.9 Liveborn child: 2.7–222.2	↔
	Pregnancy loss (clinical)	MEHP, MEHHP, or MEOHP	See above	↔
Whyatt et al. 2009 Cohort, 311 mother-infant pairs (mean age 25.5 years), United States (New York)	**Gestational age**	**ΣDEHP**	NR	↓
		MEHP	IQR: 1.8–12.8 ng/mL	↓
		MEHHP	10.3–44.4	↓
		MEOHP	8.9–35.1	↓
		MECPP	18.7–76.2	↓
Wolff et al. 2008 Cohort, 404 mother-infant pairs (mean age 24 years), United States (New York)	**Gestational age**	ΣDEHP	IQR: 0.13–0.5 µmol/L	↔
		MEHP	IQR: 2.9–14 ng/mL	↑
		MEHHP	9.5–39	↔
		MEOHP	8.3–36	↔
		MECPP	16–70	↔

Table 2-11. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Zhang et al. 2020a, 2020d Cohort, 419 females seeking fertility treatment (mean age 34.7 years), metabolites determined in preconception urine samples, United States (Massachusetts)	**Preterm birth (<37 weeks)**	**ΣDEHP**	IQR: 22.1–79.2 µmol/L (SG-adj)	↑
		MEHP	IQR: 1.2–4.0 ng/mL (SG-adj)	↑
		MEHHP	5.5–21.8	↑
		MEOHP	3.6–14.2	↑
		MECPP	10.1–35.4	↑

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; GW = gestation week; ICSI = intracytoplasmic sperm injection; IQR = interquartile range; IVF = *in vitro* fertilization; LOD = limit of detection; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NR = not reported; SG-adj = specific gravity adjusted

white women, not African American women. Additionally, Ferguson et al. (2019a, 2019b) found an interaction between preterm birth and the sum of third trimester urinary DEHP metabolites only in women with a stressful life event (e.g., job loss, serious illness, family death, relationship issues, or legal or financial problems). Other cohort studies observed either no association between exposure and preterm birth (Ferguson et al. 2019c; Hu et al. 2020; Shoaff et al. 2016) or decreased odds of preterm birth with increased exposure (Adibi et al. 2009). Two cohort studies reported increased odds of post-term (>41 weeks) birth with increased maternal urinary DEHP metabolite levels (Adibi et al. 2009; Gao et al. 2019).

In studies of gestational age as a continuous variable, no clear relationship with urinary DEHP metabolite levels was seen. Of the 10 studies that evaluated gestational age, two reported increased gestational age associated with increased urinary DEHP metabolite levels (Adibi et al. 2009; Wolff et al. 2008), one reported an association between decreasing gestational age and increasing metabolite levels (Whyatt et al. 2009), and the remaining studies reported no association (Casas et al. 2016; Ferguson et al. 2019c; Gao et al. 2017, 2019; Hu et al. 2020; Shoaff et al. 2016; Su et al. 2014). Inconsistencies among the studies may result from the varying times of urine sample collection, validity of outcome assessment, or selection or omission of important covariates. Importantly, the timing of urine sample collection may have a significant impact on a study's ability to detect an association. A systematic review of 15 studies recommends collection of samples in each trimester, standardization of sample collection to a specific time of day, and correction for specific gravity (not creatinine) to reduce intra- and within-individual variability (Yaghjyan et al. 2016).

Four studies distinguished spontaneous preterm birth (spontaneous labor or membrane rupture) from other causes of preterm birth (i.e., intrauterine growth retardation [IUGR], preeclampsia, or other maternal complications) (Ferguson et al. 2014b, 2019a, 2019b, 2019c; Hu et al. 2020). Two cohorts observed an association between spontaneous preterm birth and the sum of DEHP metabolites in urine (Ferguson et al. 2014b, 2019a, 2019b). For the Ferguson et al. (2019a, 2019b) cohort, this finding was restricted to third trimester urine levels only; however, in the study by Ferguson et al. (2014b), this association exhibited an exposure-related trend across quartiles of exposure (geometric mean across three visits), and also held true for three of the four individual metabolites measured (MEHP, MEOHP, and MECPP). Ferguson et al. (2014b) proposed that increased risk of preterm birth may be associated with pro-inflammatory activities of DEHP based on positive associations between DEHP exposure and systemic markers of inflammation and oxidative stress (Ferguson et al. 2012). In support of this proposed mechanism, follow-up studies in this birth cohort showed a positive association between maternal urinary

levels of DEHP metabolites and urinary levels of the oxidative stress marker, 8-isoprostane (Ferguson et al. 2015). Additionally, the association between urinary DEHP metabolites and spontaneous preterm birth was mediated by maternal urinary levels of 8-isoprostane using complex regression models (Ferguson et al. 2017).

Pregnancy loss, or spontaneous abortion, and/or failed live birth was evaluated in four cohort studies of pregnant women (Jukic et al. 2016; Machtinger et al. 2018; Messerlian et al. 2016b; Toft et al. 2012), two cohort studies of women receiving IVF/ICSI (Al-Saleh et al. 2019d; Deng et al. 2020), and one case-control study that measured exposure using urinary metabolites of DEHP (Mu et al. 2015a). When evaluating early (or biochemical) pregnancy loss, three studies reported increased risk of early pregnancy loss with an increase in urinary levels of one or more DEHP metabolites (Al-Saleh et al. 2019d; Messerlian et al. 2016b; Toft et al. 2012), one study observed decreased odds of early pregnancy loss with increased urinary metabolite levels (Jukic et al. 2016), and one study observed no association (Deng et al. 2020). Regarding clinical pregnancy loss, only one study observed an association with exposure to DEHP (Al-Saleh et al. 2019d).

One prospective cohort study of 132 sub-fertile females did not observe an association between preconception and prenatal urinary DEHP metabolite levels and decreased placental weight (Mustieles et al. 2019). No other studies of this endpoint were identified in the available literature.

In a case-control study of 50 cases of preeclampsia and 431 pregnancies without preeclampsia, Cantonwine et al. (2016) observed increased hazard ratios for preeclampsia with interquartile range increases in maternal urinary levels of MEHP and the sum of DEHP metabolites. No other studies of this endpoint were identified in the available literature.

Nonhuman Primates—Female Reproductive Effects. Few female reproductive studies of DEHP have been conducted in nonhuman primates. A 13-week gavage study in marmosets of unspecified age showed no significant treatment-related effects on gross or microscopic appearance of the uterus, vagina, or ovary at doses up to 2,500 mg DEHP/kg/day (Kurata et al. 1998).

Rodent Studies—Female Reproductive Effects. Two-generation studies in Wistar rats reported decreased F1 fertility and increased post-implantation loss in F0 dams after exposure to doses ≥1,040 mg/kg/day, but not ≤380 mg/kg/day (Schilling et al. 1999, 2001). Evidence of decreased growing ovarian follicles and corpora lutea in F0 and F1 females exposed to 1,088 mg/kg/day suggest that

alterations in the female reproductive system may contribute to decreased F1 fertility; however, these studies provide strong evidence for damage to the male reproductive system (discussed above). In a chronic exposure 2-generation study in Sherman rats, no changes in fertility or reproductive organ histology were observed; however, the highest dose evaluated was 200 mg/kg/day (Carpenter et al. 1953). In a 3-generation, continuous breeding study with cross-over mating, decreased fertility in the F1 and F2 generation was attributed to effects in males, with no clear evidence of decreased female fertility in the cross-over mating trial at 659 mg/kg/day (Blystone et al. 2010; NTP 2005). Additionally, no changes were observed in female reproductive organ weights or histology.

In CD-1 mice, decreased fertility in a continuous breeding study at doses ≥130 mg/kg/day was attributed to both males and females in a cross-over trial, as fertility issues were observed when females exposed at 390 mg/kg/day were mated to unexposed males or vice versa (Lamb et al. 1987; Morrissey et al. 1988; NTP 1984). In the main mating trial, decreased fertility, decreased numbers of litters/pair, decreased numbers of pups/litter, and decreased numbers of live-born pups were observed at 130 mg/kg/day, with no litters produced at 390 mg/kg/day. The combined weight of the ovaries, oviducts, and uteri of exposed females from the crossover trial was significantly decreased compared with controls. In 1-generation studies, no changes in fertility or pregnancy outcomes were observed in CD-1 or C57BL/6J x FVB mice following exposure to doses up to 0.34 or 100 mg/kg/day, respectively, for 2 weeks prior to mating through lactation (Bastos Sales et al. 2018; Cha et al. 2018).

In CD-1 mice exposed to ≥0.2 mg/kg/day for 30 days prior to mating, decreased oocyte fertilization, zygote fragmentation and arrested development, and a decreased number of preimplantation embryos were observed 24–96-hours post mating to untreated males; no effect was noted at 0.02 mg/kg/day (Parra-Forero et al. 2019). In similarly exposed female CD-1 mice that were super-ovulated, but not mated, the number of oocytes recovered was decreased at 2 mg/kg/day (Parra-Forero et al. 2019). Altered estrous cycles (increased percentage of days spent in estrus) were also observed in CD-1 mice exposed to 200 mg/kg/day for 30 days, but not at doses ≤20 mg/kg/day (Hannon et al. 2014). No dose-related changes were observed in the number of follicles in ovaries or uterine weight. Gene expression analysis showed significant alterations in genes within the PI3K pathway, which regulates early folliculogenesis, including decreased Pten at ≥20 mg/kg/day and decreased Tsc1 at 200 mg/kg/day (Hannon et al. 2014). In female B6C3F1 mice, a complete absence of corpora lutea was observed after exposure to dietary doses of approximately 7,899 mg/kg/day DEHP for 28 days; ovarian histology was not evaluated at lower doses in the study (Myers 1992a).

A 14-day study in ICR mice reported decreased oocyte maturation and decreased IVF rates at ≥0.01 and 0.04 mg/kg/day (Lu et al. 2019); however, data cannot be adequately evaluated because the statistical unit of comparison is the oocyte (not the treated animal). Due to this issue, it cannot be determined if one or two animals are driving the observed results. This study also qualitatively reported altered morphology of the primary follicle, but the dose(s) at which these effects were observed were not reported. Due inadequate data reporting and inappropriate statistical analysis, this study is not included in the LSE table.

In a series of experiments in mice, female reproductive endpoints were evaluated 0–24 months after a 10-day exposure to DEHP at doses ranging from 0.02 to 750 mg/kg/day (Chiang and Flaws 2019; Chiang et al. 2020a, 2020b; Hannon et al. 2014). The study authors conclude in all studies that there is evidence that DEHP causes reproductive effects in females; however, the conclusions are based on numerous non-dose-related changes in organ weight, folliculogenesis, estrous cyclicity, and reproductive hormone levels with little concordance between studies and evaluation timepoints. One study (Chiang and Flaws 2019) reported reduced fertility 3 months post-exposure to 0.02 mg/kg/day, but fertility effects were not observed at doses ≥0.2 mg/kg/day or earlier or immediately, 9 months, or 12 months postexposure at any dose. None of the studies provided potential rationales supporting evidence or proposed mechanisms of action for a non-monotonic response. Based on lack of clearly adverse, dose-related findings, these studies were not included in the LSE table.

In gestation-only studies, increased resorptions and post-implantation losses, and decreased uterine weights, were observed in Wistar rat dams exposed to 1,000 mg/kg/day from GD 6 to 15, but not ≤200 mg/kg/day (Hellwig et al. 1997). Vaginal hemorrhage was observed in two of nine dams exposed to 1,000 mg/kg/day. Increased post-implantation losses and decreased litter sizes were also observed in Wistar rat dams exposed to 500 mg/kg/day during gestation, but not ≤100 mg/kg/day (Dalsenter et al. 2006). In mice, gestational exposure resulted in decreased numbers of live pups/litter at doses ≥95 mg/kg/day, increased resorptions and late fetal deaths at ≥250 mg/kg/day, and complete litter losses at ≥500 mg/kg/day (Gu et al. 2016; Pocar et al. 2012; NTP 1988; Schmidt et al. 2012; Shiota and Nishimura 1982; Shiota et al. 1980; Tyl et al. 1988; Ungewitter et al. 2017). No changes in pregnancy outcomes were observed at ≤91 mg/kg/day. In numerous other studies, no changes in gestation length, litter sizes, or sex ratios were observed following gestational exposure to DEHP at doses up to 900 mg/kg/day in rats or 700 mg/kg/day in mice (Table 2-2).

In pregnant mice, exposure to DEHP on GDs 0–14 resulted in a significant 3- or 9-fold increase in serum progesterone levels at 50 and 200 mg/kg/day, respectively; serum estradiol levels were unaltered (Zhang

et al. 2020b). A significant 25% decrease in serum estradiol levels was observed on GD 12.5 in mouse dams exposed to 0.04 mg/kg/day via gavage from GD 0.5 to 19.5, compared with controls (Zhang et al. 2015). In nonpregnant mice, no exposure-related changes in serum estradiol were observed following exposure to doses up to 10 mg/kg/day for 45 days (Xie et al. 2019).

Additional studies in rodents that did not evaluate reproductive performance show limited evidence of reproductive effects in nonpregnant female mice. One gavage study in rats reported a 16–17% decrease in absolute and relative weight of the left ovary following exposure to ≥30 mg/kg/day for 13 weeks starting on PND 6; however, no changes were observed in the right ovary (Kim et al. 2018c). In other intermediate-duration oral studies, no changes in ovary weights or reproductive organ histology were observed in rats or mice at doses up to 3,000 or 2,500 mg/kg/day, respectively (Gray et al. 1977; Myers 1992b; NTP 1982; Toyosawa et al. 2001), although decreased uterine weights were observed in rats at 1,858 mg/kg/day (Myers 1992b). In chronic-duration studies, no changes in female reproductive organ histology were observed in rats at doses up to 939 mg/kg/day (David et al. 2000a; Kluwe et al. 1982a; NTP 1982). In mice, suppurative inflammation in the uterus/endometrium was observed following exposure to 1,821 mg/kg/day for 2 years, with no adverse histological effects at doses up to 1,458 mg/kg/day (David et al. 2000b; Kluwe et al. 1982b; NTP 1982). However, reduced uterus weights were also observed in female B6C3F1 mice exposed to 1,458 mg/kg/day for 2 years (David et al. 2000b).

In a study that evaluated the estrogenic activity of DEHP and other phthalate esters, DEHP did not affect the degree of vaginal epithelial cell cornification in mature ovariectomized rats following exposure to doses up to 2,000 mg/kg/day for 4 days (Zacharewski et al. 1998).

Mechanisms of Female Reproductive Toxicity. DEHP has been shown to affect various stages of mammalian folliculogenesis following *in vivo* and *in vitro* exposure. Observed effects include altered development of the primordial germ cell, impaired primordial follicle assembly, impaired oocyte survival and meiosis, cell cycle arrest and apoptosis in ovarian granulosa cells, reduced oocyte nest breakdown, acceleration of primordial follicle activation, altered follicle steroidogenesis, increased follicle atresia, and impaired growth of antral follicles (Absalan et al. 2017; Li et al. 2012b, 2016; Mu et al. 2015b; Tripathi et al. 2019; Zhang et al. 2013, 2014, 2015). Apoptosis in ovarian granulosa cells appears to result from generation of ROS leading to reduced expression of steroidogenesis genes (Cyp11a1, Cyp19A1, Star, ERβ1) (Tripathi et al. 2019). Folliculogenesis effects appear to be mediated, in part, by DEHP or MEHP binding to PPARs and/or ERs. Although the exact mechanism is unknown, binding to these receptors

appears to alter the ability of endogenous hormones to regulate normal ovarian development (Zhang et al. 2015).

Lovekamp-Swan and Davis (2003) suggested that MEHP interacts with PPARs to decrease aromatase activity and estradiol production in the ovary, resulting in decreased ovulation and reduced fertility. In *in vitro* studies, co-exposure of DEHP with an ER antagonist (ICI 182,780) reversed DEHP-mediated impairments during primordial follicle assembly (Mu et al. 2015b). Zhang et al. (2018c) demonstrated a role for the induction of autophagy in the disruption of primordial folliculogenesis by DEHP. DEHP increased the expression of autophagy-related genes and resulted in an increase in recognizable autophagosome in ovarian cell culture. Impaired oocyte maturation post-fertilization may be due to impaired DNA replication during mitosis, as the numbers of 1-cell zygotes with DNA replication were significantly decreased in DEHP exposed animals (Parra-Forero et al. 2019).

In a review by Cheon (2020), it is proposed that DEHP affects uterine histology (e.g., thickness of the endometrium, change in the number of endometrial glands) through alteration of the expression and regulation of steroid hormone receptors. One study suggests that DEHP impairs endometrial receptivity to embryo implantation, which could result in decreased fertility (Li et al. 2012c). In this study, decreased implantation was associated with elevated protein expression levels of ERα, progesterone receptor (PR), and E-cadherin in the mouse endometrium. The E-cadherin finding suggests that the MAPK and NF-κB signaling pathways may be influenced by DEHP exposure. Decreased PR has also been observed in placental cells of DEHP-exposed pregnant mice; this finding was associated with elevated serum progesterone levels and a decreased number of proliferating cells in the placenta (Zhang et al. 2020b). DEHP can also alter sterologenesis in the liver of rodents, which may have an impact on steroid-dependent functions. For example, feeding female rats DEHP at an estimated dose of 500 mg/kg/day for 13 days significantly inhibited sterologenesis from ^{14}C-mevalonate in liver and adrenal minces (Bell 1980).

Several mechanisms have been proposed to contribute to DEHP-induced pregnancy loss and preterm birth, including alteration of ovarian steroidogenesis, placental alterations, intrauterine inflammation, and vitamin D deficiency (Basak et al. 2020; Johns et al. 2017; Marie et al. 2015).

Additional mechanisms of female reproductive toxicity occurring after gestational or early postnatal exposure to DEHP are in Section 2.17 (Developmental; Mechanisms of Altered Female Reproductive Development).

Summary. Human epidemiological studies suggest potential associations between DEHP exposure and decreased serum testosterone and diminished semen quality in adult men. Available studies on fertility effects in humans are limited, but do not indicate an association between DEHP exposure and infertility. Numerous studies in rodents have shown that the mature male reproductive systems, particularly the testes, are susceptible to DEHP toxicity, and that DEHP exposure leads to decreased male fertility in both rats and mice. Limited data indicate that nonhuman primates are not susceptible or less susceptible to male reproductive toxicity following exposure to DEHP. Alterations in female reproductive systems, including decreased fertility, have been reported in animals at higher doses than those associated with male reproductive effects. Taken together, available human and animal data indicate that the adult male reproductive system is a sensitive target of DEHP toxicity.

2.17 DEVELOPMENTAL

Overview. Many human and animal studies have evaluated whether DEHP may affect development. The most studied endpoints include birth size and growth, and development of the reproductive and neurological systems. The development of the hepatic and renal systems as well as metabolic function (glucose homeostasis) have also been evaluated. In addition, meta-analyses and systematic review regarding developmental reproductive effects in male humans and animals have been conducted by NAS. Studies discussed in this section include those with prenatal, early postnatal, and/or pre-pubescent exposure. For studies that exposed animals both prior to and through sexual maturation into adulthood (e.g., multigenerational studies), endpoints evaluated after sexual maturation are in the respective organ system section of this profile (e.g., reproductive), while endpoints evaluated prior to sexual maturation are below.

Epidemiology Studies—Birth Size and Growth. Measures of birth size evaluated in epidemiological studies of DEHP include birth length, birth weight, and head and chest circumference (Table 2-12). Findings were inconsistent among the 15 studies that met inclusion criteria (Appendix B). Zhao et al. (2014) observed exposure-related increases in the odds of IUGR across tertiles of maternal urinary DEHP metabolites in a case-control study in China (42 infants with IUGR and 84 controls matched on maternal age). A relationship between lower birth weight and higher urinary levels of MEHHP and MEOHP, especially among male infants, was also observed. In contrast, Sathyanarayana et al. (2016b) reported increased birth weight in female infants, but not male infants, with increasing DEHP metabolite levels in

Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Bloom et al. 2019a, 2019b Cohort, 310 mother-infant pairs (152 African American and 158 White mothers; mean age 27.6 years), urinary metabolites measured at 18–22 weeks (1st visit) and 24–32 weeks (2nd visit); United States (South Carolina)	**Small for gestational age**	**ΣDEHP**	All women (1st visit): IQR: 33.5–92.0 nmol/L (SG-adj)	↔
			All women (2nd visit): 37.8–81.7	↔
			African American (1st visit): 21.5–69.4	↔
			African American (2nd visit): NR	↔
			White (1st visit): 22.1–52.1	↓
			White (2nd visit): NR	↔
		MEHP	All women (1st visit): IQR: 1.5–5.3 ng/mL (SG-adj)	↔
			All women (2nd visit): 1.4–4.5	↔
			African American (1st visit): 1.0–4.1	↔
			African American (2nd visit): NR	↔
			White (1st visit): 0.8–2.6	↓
			White (2nd visit): NR	↔
		MEHHP	All women (1st visit): 3.5–9.1	↔
			All women (2nd visit): 3.5–8.1	↔
			African American (1st visit): 2.5–7.9	↔
			African American (2nd visit): NR	↔
			White (1st visit): 2.8–6.1	↔
			White (2nd visit): NR	↔
		MEOHP	All women (1st visit): 4.1–12.2	↔
			All women (2nd visit): 4.7–10.9	↑
			African American (1st visit): 2.1–5.8	↔
			African American (2nd visit): NR	↔
			White (1st visit): 2.1–5.0	↔
			White (2nd visit): NR	↔

Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
	Birth weight for gestational age Z-score or low birth weight	ΣDEHP, MEHP or MEOHP	All women, African American women, White women (both time points): see above	↔
		IQRs were estimated from graphically presented data using GrabIt! software.		
Casas et al. 2016 Cohort, 657 pregnant women (age ≥16 years), Spain	Birth length; birth weight; or head circumference	ΣDEHP	Range: 26.5–1,670 µg/g Cr	↔
Chiu et al. 2018a, 2018b Cohort, 300 mother-infant pairs (mean age 34.6 years), United States (Massachusetts)	Birth weight	MEHP	IQR: 1.3–4.7 µg/L (SG-adj)	↔
		MEHHP	6.5–21.9	↔
		MEOHP	4.8–16.2	↔
		MECPP	10.7–32.7	↔
Gao et al. 2017 Cohort, 3,103 mother-infant pairs (mean age 26.4 years), China	Birth weight, Birth length, or Head or chest circumference	ΣDEHP	NR	↔
		MEHP	25^{th}–95^{th} percentile: 1.34–13.86 µg/g Cr	NR
		MEHHP	3.01–20.19	NR
		MEOHP	4.32–23.05	NR
Goodrich et al. 2019 Cohort, 56 mother-infant pairs (mean age 31.5 years), United States (Michigan)	Birth weight or Fenton Z-score (standardized birth weight for gestational age and sex)	ΣDEHP	IQR: 6.88–34.52 µg/L	↔
		MEHP	<LOD–4.24	↔
		MEHHP	2.45–11.63	↔
		MEOHP	1.21–6.08	↔
		MECPP	2.26–12.83	↔
Kim et al. 2016a Cohort, 128 mother-infant pairs including 65 boy infants (mean age 33 years) and 63 girl infants (mean l age 34 years), Korea	**Birth length**	**ΣDEHP**	NR	All: ↔ **Boys: ↑** Girls: ↔
		MEHHP	Infant (first urine) IQR: 3.21–11.87 ng/mL (SG-adj)	All: ↔ **Boys: ↑** Girls: ↔

Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
		MEOHP	1.51–6.50	All: ↔ **Boys:** ↑ Girls: ↔
	Ponderal index at birth	**ΣDEHP, MEHHP, MEOHP**	See above	All: ↔ **Boys:** ↓ Girls: ↔
	Birth weight or head circumference	ΣDEHP, MEHHP, or MEOHP	See above	↔
Messerlian et al. 2017a Cohort, 364 mother-infant pairs including 208 conceived via IVF (mean age 35.0 years) and 156 non-IVF (mean age 34.3 year) and 195 male partners of sub-fertile couples including 119 conceived via IVF (mean age 36.0 years) and 76 non-IVF (mean age 35.6 years), United States (Massachusetts)	**Birth weight (IVF conceived)**	**ΣDEHP**	**Paternal, preconception**: IQR: 32.4–136.6 ng/mL (SG-adj)	↓
			Maternal, prenatal: 25.3–75.5	↓
		MEHP	Paternal, preconception: 1.4–7.2	↔
			Maternal, prenatal: 1.4–4.7	↓
		MEHHP	**Paternal, preconception:** 8.5–40.8	↓
			Maternal, prenatal: 6.6–21.9	↓
		MEOHP	**Paternal, preconception:** 5.4–22.7	↓
			Maternal, prenatal: 4.8–15.9	↓
		MECPP	**Paternal, preconception:** 14.8–66.1	↓
			Maternal, prenatal: 10.7–33.3	↓
	Birth weight (non-IVF conceived)	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	Paternal, preconception: see above	↔
			Maternal, prenatal: see above	↔
		Birth weight was not associated with maternal preconception metabolite levels in either IVF or non-IVF infants.		
Sathyanarayana et al. 2016b Cohort, 753 mother-infant pairs including 369 boys and 384 girls (age ≥18 years), United States (California, Minnesota, New York, Washington)	**Birth weight**	**ΣDEHP**	NR	Boys: ↔ **Girls**: ↑
		MEHP	IQR: 1.37–4.35 ng/mL	Boys: ↔ **Girls**: ↑
		MEHHP	4.35–12.77	Boys: ↔ **Girls**: ↑

Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
		MEOHP	3.13–8.70	Boys: ↔ **Girls**: ↑
		MECPP	5.90–15.95	Boys: ↔ **Girls**: ↑
Shoaff et al. 2016 Cohort, 368 mother-infant pairs (age ≥18 years), United States (Ohio)	Birth weight	ΣDEHP	16 weeks: IQR: 0.14–0.72 nmol/mL 26 weeks: 0.10–0.52	↔
Su et al. 2014 Cohort, 130 mother-infant pairs (maternal age NR), Taiwan	Birth length, birth weight, or head circumference	ΣDEHP	IQR: 42.28–60.83 µg/g Cr	↔
		MEHP	14.56–20.19	↔
		MEHHP	5.49–10.53	↔
		MEOHP	10.05–17.58	↔
Tsai et al. 2018a, 2018b Cohort, 112 mother-infant pairs from Cathy General Hospital (CGH) group (potentially exposed to tainted food; mean age 31.93 years)) and 245 mother-infant pairs from Taiwan Maternal and Infant Cohort (TMIC) (became pregnant after the tainted food products were removed from the market; mean age 31.99 years), Taiwan	Birth weight or height or head or chest circumference	ΣDEHP	1st trimester: CGH, IQR: 178.16–463.36 µg/g Cr TMIC: 103.19–208.10	↔
			2nd trimester: CGH: 210.76–471.14 TMIC: 48.18–151.75	↔
			3rd trimester: CGH: 202.14–513.31 TMIC: 94.01–220.78	↔
		MEHP	1st trimester: CGH: 14.85–46.78 TMIC: 2.98–8.82	↔
			2nd trimester: CGH: 21.56–43.89 TMIC: 0.24–5.57	↔
			3rd trimester: CGH: 18.13–51.35 TMIC: 1.58–6.93	↔

Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
		MEOHP	1st trimester: CGH: 6.73–21.70 TMIC: 5.47–12.83	↔
			2nd trimester: CGH: 7.44–23.66 TMIC: 2.13–8.83	↔
			3rd trimester: CGH: 8.44–23.98 TMIC: 5.95–15.34	↔
		MEHHP	1st trimester: CGH: 9.80–29.04 TMIC: 7.87–16.58	↔
			2nd trimester: CGH: 8.91–28.51 TMIC: 2.60–10.22	↔
			3rd trimester: CGH: 9.25–30.13 TMIC: 7.07–18.95	↔
		MECPP	1st trimester: CGH: 13.57–37.76 TMIC: 10.68–22.76	↔
			2nd trimester: CGH: 14.58–36.06 TMIC: 6.75–18.43	↔
			3rd trimester: CGH: 14.48–41.90 TMIC: 11.22–24.67	↔
Wolff et al. 2008 Cohort, 404 mother-infant pairs (mean age 24 years), United States (New York)	Birth length, birth weight, or head circumference	ΣDEHP	IQR: 0.13–0.5 µmol/L	↔
		MEHP	IQR: 2.9–14 ng/mL	↔
		MEHHP	9.5–39	↔
		MEOHP	8.3–36	↔
		MECPP	16–70	↔

Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Zhang et al. 2018d Cohort, 3,103 mother-infant pairs including 74 mother-infant pairs with low birth weight infants (<2,500 g; 35 boys, 39 girls; mean age 26.07 years), 2,783 mother-infant pairs with normal birth weight infants (2,500–4,000 g; 1,391 boys, 1,383 girls; mean age 26.39 years), and 246 mother-infant pairs with high birth weight infants (>4,000 g; 138 boys, 107 girls; mean age 26.46 years), China	**Birth weight (low birth weight infants)**	**ΣDEHP**	1st trimester: IQR: 2.40–3.28 ng/mL 2nd trimester: 2.43–3.42 3rd trimester: 1.99–2.92	**All:** ↓ **Boys:** ↓ **Girls:** ↓
		MEHP	1st trimester: 0.45–1.57 2nd trimester: 0.86–1.97 3rd trimester: 0.36–1.57	**All:** ↓ **Boys:** ↓ Girls**:** ↔
		MEHHP	1st trimester: 1.22–2.19 2nd trimester: 1.31–2.42 3rd trimester:0.84–1.87	**All:** ↓ **Boys:** ↓ Girls**:** ↔
		MEOHP	1st trimester: 1.55–2.46 2nd trimester: 1.49–2.47 3rd trimester: 1.07–2.04	**All:** ↓ **Boys:** ↓ Girls**:** ↔
	Birth weight (normal birth weight infants)	ΣDEHP, MEHHP, or MEOHP	See above	↔
		MEHP	See above	All: ↔ **Boys:** ↑ Girls**:** ↔
	Birth weight (high birth weight infants)	**ΣDEHP**	See above	All: ↔ Boys: ↔ **Girls:** ↓
		MEHP	See above	**All:** ↓ Boys: ↔ Girls**:** ↔
		MEHHP	See above	**All:** ↓ Boys: ↔ **Girls:** ↓
		MEOHP	See above	↔
	Birth weight (all infants)	ΣDEHP, MEHP, MEHHP, or MEOHP	See above	↔

Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Zhao et al. 2014 Case-control, 42 IUGR infants and 84 controls (maternal age NR), China	**Birth weight**	ΣDEHP	All: IQR: 13.6–46.3 ng/mL Cases: 16.4–54.5 Controls: 9.3–41.5	↔
		MEHP	All: 1.5–17.4 Cases: 3.5–16.7 Controls: 0.7–17.4	↔
		MEHHP	All: 3.9–19.2 Cases: 6.6–29.8 Controls: 3.2–15.8	↓
		MEOHP	All: 1.7–9.7 Cases: 2.4–15.0 Controls: 1.4–6.4	↓
	Birth length	ΣDEHP, MEHP, MEHHP, MEOHP	See above	↔
	IUGR	ΣDEHP, MEHP, or MEOHP	See above	↔
		MEHHP	See above	↑
Zhu et al. 2018 Cohort, 1,002 mother-infant pairs (525 boys, 477 girls; mean age 28.7 years), China	**Birth weight or birth weight Z-score**	**ΣDEHP**	IQR: 104–255 nmol/g Cr	↑ **(boys)** ↔ (girls)
		MEHHP	IQR: 10.0–25.4 µg/g Cr	↔
		MEOHP	8.90–23.2	↑ **(boys)** ↔ (girls)
		MECPP	11.4–27.6	↑ **(boys)** ↔ (girls)
	Birth length	ΣDEHP, MEHHP, MEOHP, or MECPP	See above	↔

Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
	Ponderal index (birth weight/birth length)	ΣDEHP, MEHHP, or MEOHP	See above	↔
		MECPP	See above	↑ **(boys)** ↔ (girls)

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; IQR = interquartile range; IUGR = intrauterine growth retardation; IVF = *in vitro* fertilization; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NR = not reported; SG-adj = specific gravity adjusted

maternal urine, while Zhu et al. (2018) reported increased birth weight and ponderal index (birth weight/birth length) in male infants, but not female infants, with increasing DEHP metabolite levels in maternal urine. Kim et al. (2016a) reported an increase in birth length with a corresponding decrease in ponderal index in boys, but not girls, with increasing DEHP metabolite levels in infant first urine.

In a cohort study that stratified analysis by birth weight status (low, normal, or high birth weight), maternal urine DEHP metabolite levels were associated with decreasing birth weight in low-birth weight male and female infants, increased birth weight in normal weight male infants, and decreased birth weight in high weight female infants (Zhang et al. 2018d). Among sub-fertile couples, birth weight in babies conceived via IVF was negatively associated with paternal preconception and maternal prenatal urinary DEHP metabolite levels (Messerlian et al. 2017a). Bloom et al. (2019a, 2019b) reported decreased risk of small for gestational age in white, but not African American, women with increasing sum DEHP or MEHP metabolites; when both races were combined, an increased risk of small for gestation age was observed with increasing urinary MEOHP levels. Other studies did not observe an association between DEHP exposure and measures of birth size (Casas et al. 2016; Chiu et al. 2018a, 2018b; Gao et al. 2017; Goodrich et al. 2019; Shoaff et al. 2016; Su et al. 2014; Tsai et al. 2018a, 2018b; Wolff et al. 2008).

Epidemiological studies evaluating the effects of prenatal exposure to DEHP and growth or obesity parameters in children have also not shown consistent results, as shown in Table 2-13. In general, no association was observed between maternal metabolite levels and BMI measured at ages ranging from 2 to 14 years of age (Agay-Shay et al. 2015; Buckley et al. 2016a; Harley et al. 2017; Heggeseth et al. 2019a, 2019b; Shoaff et al. 2017a, 2017b; Vafeiadi et al. 2018a, 2018b). Two studies reported decreased BMI with an increased prenatal DEHP exposure (Lee et al. 2020; Valvi et al. 2015). Lee et al. (2020) observed decreased BMI in 6-year-old girls, but not boys, with increased MEHHP in maternal urine. In contrast, Valvi et al. (2015) observed decreased BMI in 4- and 7-year-old boys, but not girls, with increased ΣDEHP in maternal urine; this association was not observed at 1 year of age. Kim et al. (2016a) reported increased odds of higher growth (increase in BMI z-score more than the 50^{th} percentile change between birth and 3 months of age) with higher levels of MEHHP and MEOHP in newborn urine. However, birth weight and length at 3 months of age were obtained by telephone interview with mothers rather than clinical examination and measurement by a physician, rendering the growth estimates uncertain.

Fewer data are available for other measures of growth or obesity (Table 2-13). One study reported increased odds of being overweight or obese at 12 years of age when DEHP metabolite levels were

Table 2-13. Summary of Epidemiological Studies of Prenatal DEHP Exposure and BMI, Waist Circumference, and Measures of Adiposity

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Agay-Shay et al. 2015 Cohort, 470 children assessed at age 7 years, Spain	BMI	MEHP	Maternal range: 1.8–266.9 µg/g Cr	↔
		MEHHP	5.3–503.4	↔
		MEOHP	4.1–378.3	↔
		MECPP	7.7–718.9	↔
Buckley et al. 2016a Cohort, 707 children assessed at age 4–7 years, United States (New York and Ohio)	BMI	ΣDEHP	Maternal IQR: 0.128–0.562 µmol/L	↔ Maresca et al. (2016) evaluated the same outcome in a subset of this cohort; no significant association was observed.
Buckley et al. 2016b Cohort, 180 children assessed at age 4–9 years, United States (New York)	Percent fat mass	ΣDEHP	Maternal IQR: 125–530 nmol/L	↔
Harley et al. 2017 Cohort, 345 children assessed at age 5–12 years, United States (California)	**Overweight or obese**	**ΣDEHP**	Maternal IQR: 0.1–0.3 µmol/L	5–10.5 years: ↔ **12 years**: ↑
	Waist circumference	**ΣDEHP**	See above	**5 years**: ↑ 7–12 years: ↔
	BMI or percent body fat	ΣDEHP	See above	5–12 years: ↔
Heggeseth et al. 2019a, 2019b Cohort, 335 children assessed four or more times between age 2 and 14 years, United States (California)	BMI	MEHP	Maternal IQR: 2.1–6.9 ng/mL	↔
		MEHHP	8.2–26.3	↔
		MECPP	15.6–41.4	↔
		MEOHP	6.4–19.375	↔
Kim et al. 2016a Cohort, 128 infants assessed at 3 months, Korea	**BMI Z-score (change from birth 3 months)**	**ΣDEHP**	NR	↑
		MEHHP	Infant (first urine) IQR: 3.21–11.87 ng/mL	↑
		MEOHP	1.51–6.50	↑

Table 2-13. Summary of Epidemiological Studies of Prenatal DEHP Exposure and BMI, Waist Circumference, and Measures of Adiposity

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Lee et al. 2020 Cohort/Cross-sectional, 481 children (255 boys, 226 girls) assessed at age 6 years, South Korea	**BMI Z-score**	ΣDEHP	Maternal): IQR: 0.06–0.17 nmol/g Cr	↔
			Child (6 years): 0.23–0.47	↔
		MEHHP	Maternal: IQR: 9.4–26.5 µg/g Cr	All: ↔ Boys: ↔ **Girls:** ↓
			Child: 39.8–80.3	↔
		MEOHP	Maternal: 10.4–24.7	↔
			Child: 26.3–55.7	↔
	Percent body fat, Fat mass index (kg/m^2)	ΣDEHP, MEHHP, or MEOHP	Maternal: see above	↔
			Child: see above	↔
Maresca et al. 2016 Cohort, 424 children assessed at age 7 years, United States (New York)	Waist circumference	ΣDEHP	Maternal GM (GSD): 292.89 (3.24) nmol/L	↔
	Percent body fat	ΣDEHP	See above	↔
Shoaff et al. 2017a, 2017b Cohort/Cross-sectional, 219 children assessed at age 8 years, United States (Ohio)	**Percent body fat**	**ΣDEHP** (MEHP, MEHHP, MEOHP, MECPP)	Maternal: mean (range): 144.76 (6.09–2,408.59) ng/mL	↔
			Child (1 year): 161.17 (4.23–4,319.71)	↓
			Child (2 years): 176.20 (5.52–3,099.46)	↔
			Child (3 years): 191.22 (5.08–191.22)	↔
			Child (4 years): 176.17 (2.66–1,919.49)	↔
			Child (5 years): 106.20 (5.20–1,395.99)	↑

Table 2-13. Summary of Epidemiological Studies of Prenatal DEHP Exposure and BMI, Waist Circumference, and Measures of Adiposity

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
			Child (8 years): 87.46 (4.47–1,274.77)	↔
	Waist circumference	**ΣDEHP**	Maternal, child (1, 2, 4, or 8 years): see above	↔
			Child (5 years): see above	↑
	BMI Z-score	ΣDEHP	Maternal, child (any age)	↔
Vafeiadi et al. 2018a, 2018b Cohort/Cross-sectional, 500 children (279 boys, 221 girls) assessed at age 4–6 years, Greece	**Waist Circumference**	**ΣDEHP** (MEHP, MEHHP, MEOHP)	Maternal: IQR: 0.1–0.2 µmol/g	↔
			Child (4 years): 0.2–0.5	All: ↔ **Boys:** ↓ Girls: ↔
	Waist-to-height ratio or skinfold thickness (sum of subscapular, triceps, suprailiac, quadriceps)	**ΣDEHP**	Maternal: see above	↔
			Child: see above	All: ↔ Boys: ↔ **Girls:** ↑
	BMI Z-score	ΣDEHP	Maternal or child: see above	↔

Table 2-13. Summary of Epidemiological Studies of Prenatal DEHP Exposure and BMI, Waist Circumference, and Measures of Adiposity

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Valvi et al. 2015 Cohort, 391 children (205 boys, 186 girls) assessed at age 1, 4, and 7 years, Spain	**BMI (4 or 7 years)**	**ΣDEHP**	Maternal range: 64.9–139 µg/g Cr (as MEHP)	All: ↔ **Boys**: ↓ Girls: ↔
	BMI (1 year or all years)	ΣDEHP	See above	↔

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; BMI = body mass index; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; GSD = geometric standard deviation; IQR = interquartile range; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NR = not reported

doubled in maternal urine; however, sensitivity analysis indicated that maternal BMI influenced these results (Harley et al. 2017). A positive association was also reported between waist circumference z-score and maternal urinary DEHP levels at 5 years of age, but not at 7–12 years (Harley et al. 2017). No associations were observed between percent body fat at 9–12 years and maternal urinary DEHP levels. Maternal urinary DEHP levels were not associated with percent body fat/mass, fat mass index, or waist circumference in other studies (Lee et al. 2020; Maresca et al. 2016; Shoaff et al. 2017a, 2017b; Vafeiadi et al. 2018a, 2018b). When urinary metabolites were measured in children's urine, Shoaff et al. (2017a, 2017b) observed a negative association between percent body fat at 8 years of age and DEHP metabolite in urine collected at 1 year, while a positive association was observed for percent body fat and waist circumference at 8 years of age with DEHP metabolites in urine collected 5 years. In another cohort, urinary metabolite levels at 4 years were negatively associated with waist circumference in boys at 4–6 years, but not girls, and positively associated with waist-to-height ratio and skinfold thickness in girls at 4–6 years, but not boys (Vafeiadi et al. 2018a, 2018b).

Animal Studies—Fetotoxicity, Teratology, and Physical Growth and Development. A single inhalation study evaluated fetal skeletal and visceral effects in GD 20 offspring of female Wistar rats exposed to 0.6–21 ppm for 6 hours/day during the period of organogenesis (GDs 6–15) (Merkle et al. 1988). Skeletal and visceral effects were classified as retardations (delays in development), variations (changes that regularly occurred), or anomalies (changes that progressed beyond the degree of retardations and variations). No exposure-related skeletal retardations, variations, or anomalies or visceral variations or anomalies were observed. However, there was a statistically significant increase in the percent of litters with visceral retardations at 21 ppm, identified as "mainly" renal pelvis dilatations by the study authors (incidence data not reported). In similarly exposed dams that were allowed to deliver, no change was observed in offspring survival, growth, or development (Merkle et al. 1988).

In oral studies, increased fetal and neonatal mortality was observed in rats and mice following developmental exposure to DEHP. Fetal deaths were generally associated with maternal doses ≥340 mg/kg/day in rats and ≥95 mg/kg/day in mice (Hellwig et al. 1997; Nakamura et al. 1979; NTP 1988; Schilling et al. 1999, 2001; Tanaka 2002; Tomita et al. 1982a; Ungewitter et al. 2017; Yagi et al. 1980). However, one study did not report an increase in fetal deaths in mice following maternal exposure to doses up to 200 mg/kg/day from GD 0 to 17 (Shen et al. 2017).

Several studies reported malformations and variations following gestational exposure to similar doses. In Wistar rats, maternal exposure to 1,000 mg/kg/day on GDs 6–15 increased the incidence of fetuses with

external, soft tissue, or skeletal malformations in the tail, brain, urinary tract, gonads, vertebral column, and/or sternum (Hellwig et al. 1997). Variations and skeletal retardations were also increased at 1,000 mg/kg/day. No teratogenic effects were observed at maternal doses of 200 mg/kg/day.

Early postnatal exposure on PNDs 3–23 in Sprague-Dawley rats resulted in an increased incidence of developmental malformations in the lung parenchyma at 600 mg/kg/day (Camacho et al. 2020). In CD-1 mice exposed throughout gestation, a significant increase in malformations of the external viscera and skeleton was apparent at maternal doses ≥91 mg/kg/day (Tyl et al. 1988). Specific abnormalities included protrusion of the eyeball, exencephaly, blood vessel abnormalities, fused or branched ribs, misaligned and fused thoracic vertebrae, and tail malformations. No adverse effects were seen at a maternal dose of 44 mg/kg/day. In ICR mice, 25.8% of fetuses were malformed following exposure to a maternal dose of 341 mg/kg/day from GD 1 to 18; observed malformations included club foot, exencephaly, open eyelids, tail anomalies, myeloschisis, gastroschisis, and generalized edema (Shiota and Nishimura 1982). No fetal malformations were observed in controls or low-dose animals (85 mg/kg/day), and only 5% of fetuses were malformed at 170 mg/kg/day (Shiota and Nishimura 1982). In C57Bl/6 × B6129S4 mouse offspring, the total number of malformations was increased following maternal exposure to 250 mg/kg/day from GD 7 to 16; the most common defects were limb malformations and exencephaly (Ungewitter et al. 2017).

No changes in neonatal survival, external malformations, or acquisition of early postnatal developmental landmarks (e.g., eye opening, incisor eruption, pinna detachment) were observed in Sprague-Dawley rats following maternal exposure to doses up to 300 mg/kg/day from GD 8 to PND 21 (Nardelli et al. 2017). No gross malformations were observed in offspring of CD-1 mice exposed to doses up to 100 mg/kg/day from GD 11 to 19 (Maranghi et al. 2010). Acquisition of developmental landmarks was not altered in CD-1 mice following maternal exposure to 95 mg/kg/day from GD 0 to 17 (NTP 1988).

Numerous studies reported body weight effects in rats following developmental exposure to DEHP; however, findings are inconsistent among species, strains, and studies. Following gestation-only exposure, decreases in pup body weight ≥10% were observed in Sprague-Dawley rats at doses ≥10 mg/kg/day (Chen et al. 2010) and ≥37.5 mg/kg/day (Piepenbrink et al. (2005); however, Vo et al. (2009a) did not observe decreased body weights until doses of 500 mg/kg/day. Findings in Sprague-Dawley rats following gestation plus lactation exposure were more consistent with the Vo et al. (2009a) study, reporting no body weight changes in offspring until maternal doses ≥447 mg/kg/day (Andrade et al. 2006a, 2006c; Blystone et al. 2010; Grande et al. 2006, 2007; Gray et al. 2009; Kobayashi et al. 2006;

NTP 2005). Consistent with this, body weight decreases in Sprague-Dawley neonates following direct exposure on PNDs 3–7 or 3–23 were only observed at 600 mg/kg/day (Camacho et al. 2020). Similarly, decreased offspring body weight in Long-Evans rats was only observed at 750 mg/kg/day, not at 10 mg/kg/day (Lin et al. 2009).

Most studies in Wistar rats also reported no changes in offspring body weight following gestational and/or lactational exposure to maternal doses up to 700 mg/kg/day (Carbone et al. 2010, 2012; Dalsenter et al. 2006; Schilling et al. 1999, 2001; Venturelli et al. 2015). However, a few studies reported decreased offspring weights following maternal exposure. One study reported decreased birth weights at maternal doses ≥300 mg/kg/day (Christiansen et al. 2010). Other studies reported decreased postnatal body weights at maternal doses ≥1 mg/kg/day (measured on PNDs 9–22; Parsanathan et al. 2019) or ≥10 mg/kg/day (measured on PND 80; Rajagopal et al. 2019a). Additionally, two very low dose studies reported decreased offspring weight, body fat percentage, and adipocyte size at maternal doses ≥0.25 mg/kg/day during gestation and lactation (Lin et al. 2011; Wei et al. 2012).

Gestational studies in mice showed more consistent effects, with decreased offspring body weights in most studies at ≥191 mg/kg/day, but generally not at doses ≤100 mg/kg/day (Maranghi et al. 2010; NTP 1988; Shiota et al. 1980; Shiota and Nishimura 1982; Tyl et al. 1988; Ungewitter et al. 2017). Similarly, decreased offspring body weight and abdominal fat were observed in mouse offspring following gestational plus lactation exposure to maternal doses ≥0.05 mg/kg/day (Pocar et al. 2012; Tanida et al. 2009). One gestational study also reported decreased fetal body weight and crown-rump length at maternal doses ≥50 mg/kg/day during gestation (Shen et al. 2017).

In contrast, *increased* F1 offspring body weight and visceral adipose tissue were reported in 1-generation studies at doses ≥0.05 mg/kg/day (Fan et al. 2020; Schmidt et al. 2012). However, other 1-generation studies report a lack of body weight effects in offspring at maternal doses up to 180.77 mg/kg/day (Bastos Sales et al. 2018; Tanaka 2002). Similarly, no changes in body weight or visceral or inguinal adipose tissue were observed in postnatal week (PNW) 22 mouse offspring following maternal exposure to 0.05 or 500 mg/kg/day throughout gestation and lactation followed by high-fat diet consumption for 19 weeks, compared with unexposed high-fat diet controls (Hunt et al. 2017). Due to use of a high-fat diet, this study was not included in the LSE table.

In female weanling Wistar rats, an approximate 10% decrease in terminal body weight was observed following inhalation exposure to DEHP at 1.6 ppm for 6 hours/day, 5 days/week for the first 9 weeks

post-weaning (Ma et al. 2006). However, no body weight effects were observed in young male or female Wistar rats exposed to concentrations up to 1.6 ppm for the first 3–8 weeks post-weaning (Kurahashi et al. 2005; Ma et al. 2006). Venturelli et al. (2015) also did not observe body weight effects in Wistar rats exposed to doses up to 75 mg/kg/day for 30 days post-weaning. In weanling Long-Evans rats, a 13% decrease in body weight was observed following exposure to 750 mg/kg/day for 28 days, but not at 500 mg/kg/day for 14 or 28 days (Ge et al. 2007). Similarly, no body weight effects were observed in young Sprague-Dawley rats exposed to doses up to 500 mg/kg/day for 14–15 days post-weaning (Vo et al. 2009b; Zhang et al. 2018a).

Unspecified body weight decreases and increased mortality were observed in neonatal and weanling rats exposed to ≥1,000 mg/kg/day DEHP via gavage for 5 days (Dostal et al. 1987). Similarly, a 14-day dietary study reported a >15% decrease in body weight in sexually immature male and female F344 rats at ≥5,700 and 6,200 mg/kg/day, respectively, and male and female B6C3F1 mice at ≥4,900 and 11,000 mg/kg/day, respectively (NTP 1982).

In nonhuman primates exposed post-weaning, no exposure-related body weight effects were observed in sexually immature Cynomolgus monkeys exposed to 500 mg/kg/day for 14 days (Pugh et al. 2000) or marmoset monkeys exposed to doses up to 2,500 mg/kg/day for 65 weeks from weaning until sexual maturation (Tomonari et al. 2006).

Mechanisms of Fetotoxicity and Altered Growth. Several mechanisms have been proposed to contribute to DEHP-induced low birth weight and IUGR, including alteration of ovarian steroidogenesis, thyroid dysfunction, placental alterations, and intrauterine inflammation (Basak et al. 2020; Marie et al. 2015; Shen et al. 2017; Yu et al. 2018).

Developmental exposure to DEHP may contribute to obesity later in life via disruption of adipose tissue homeostasis. *In vitro* exposure of mouse embryonic preadipocytes to MEHP resulted in PPARγ activation, perturbation of PPARγ-induced regulators of adipogenesis and lipogenesis, and increased adipocyte differentiation (Hao et al. 2012). Perturbation of PPARγ-induced regulators of adipogenesis and lipogenesis was also observed in PND 60 mice following gestational and lactational exposure to MEHP, along with increased body and fat pad weight, increased serum cholesterol, increased triacylglycerol, and increase glucose levels (Hao et al. 2012). Specifically, DEHP exposure may result in increased adipocyte maturation via proliferating cell nuclear antigen (PCNA) phosphorylation (Hunt et al. 2017). *In vitro* studies confirm the DEHP stimulates adipogenesis in mouse embryo fibroblasts

expressing wild-type PCNA, but not in mouse embryos expressing mutated PCNA (which blocks phosphorylation) (Hunt et al. 2017).

Animal Studies—Liver System Development. As observed in the adult rodent, evidence of hepatomegaly was also observed in young animals following developmental exposure. As discussed in detail in Section 2.9 (Hepatic effects), increased liver weight without histological evidence of hepatobiliary damage is not considered adverse or relevant for human risk assessment unless at least two of the following are observed: (1) 2–3 times increase in ALT levels; (2) biologically significant change in other biomarkers of hepatobiliary damage (ALP, AST, GGT, etc.); or (3) biologically significant change in another clinical pathology marker indicating liver dysfunction (Hall et al. 2012). Therefore, evidence of increased liver weight alone is not used as a basis for a LOAEL.

In nonhuman primates, no histopathological changes in liver histology, changes in hepatic serum enzymes, evidence of liver enlargement, or peroxisomal proliferation were observed in sexually immature Cynomolgus monkeys exposed to 500 mg/kg/day via gavage for 14 days (Pugh et al. 2000) or marmoset monkeys exposed to doses up to 2,500 mg/kg/day for 65 weeks from weaning at 3 months to sexual maturity at 18 months (Tomonari et al. 2006).

There is some evidence that hepatic cellular alterations are occurring in the developing animal following DEHP exposure. However, at low DEHP doses and/or short exposure durations, these alterations appear to be reversible. Exposure for longer durations and/or to higher DEHP dose levels results in elevated liver weights accompanied by histological changes. Reversible increases in liver weights (partially recovered by PND 56) and reversible subendothelial edema of the centrilobular vein and portal space (recovered by PND 42) were seen in offspring of Long-Evans rat dams exposed to DEHP at ≥3 mg/kg/day during all of gestation and lactation (Arcadi et al. 1998). Reversible liver lesions, including pyknotic nuclei and hepatocyte vacuolation, were also observed in PND 21 offspring of CD-1 mice exposed to doses ≥25 mg/kg/day from GD 11 to 19 (Maranghi et al. 2010). Decreased glycogen storage was also observed. These effects were no longer evident at PND 35. No changes in liver histology were observed in male PND 7 Sprague-Dawley rats following direct exposure to doses up to 600 mg/kg/day from PND 3 to 7, though relative liver weights were increased at ≥300 mg/kg/day (Camacho et al. 2020). With continued exposure from PND 3 to 23, relative liver weights were only increased at 600 mg/kg/day; this was accompanied by hepatocellular hypertrophy.

In a gestational/lactational exposure study in Sprague-Dawley rats, significant increases in liver weights were observed in offspring at PND 1 at maternal doses ≥135 mg/kg/day, but not at weaning or during adulthood at maternal doses up to 405 mg/kg/day (Andrade et al. 2006a, 2006c; Grande et al. 2006, 2007). Similarly, no exposure-related changes in liver weights were observed in Sprague-Dawley rat offspring at PND 21 or 63 following maternal exposure to doses up to 400 mg/kg/day from GD 6 to PND 20 (Kobayashi et al. 2006) or at PND 3, 8, or 21 following maternal exposure to doses up to 300 mg/kg/day from GD 8 to PND 21 (Nardelli et al. 2017). In a 2-generation study in Wistar rats, increased liver weights were observed in F1 and F2 pups on PND 21 following exposure to ≥113 mg/kg/day (lowest dose tested) (Schilling et al. 2001). No exposure-related changes were observed in Wistar rat offspring on PND 16 following maternal exposure to doses up to 900 mg/kg/day from GD 7 to PND 16 (Christiansen et al. 2010). Measures of liver function and liver histology were not assessed in these studies. As discussed in Section 2.9 (Hepatic), the biological relevance of elevated liver weight in the absence of altered function or histology is unclear.

Liver weight was significantly elevated in adult male rat offspring following gestational, lactational, and direct post-lactational exposure to DEHP through PND 65 at doses ≥100 mg/kg/day, but not at doses up to 33 mg/kg/day (Gray et al. 2009). Elevated liver weight at PND 65 was not observed if DEHP exposure ceased at weaning (no direct exposure).

Significant increases in serum ALP, AST, and ALT were observed in male PND 80 Wistar rat offspring following maternal exposure to DEHP at doses ≥10 mg/kg/day from GD 9 to PND 21 (Rajagopal et al. 2019a). Liver weight and histology were not assessed. In another study, male PND 92 Wistar rat offspring showed decreased serum triglycerides and cholesterol following lactational exposure to ≥7.5 and 75 mg/kg/day, respectively (Venturelli et al. 2015). In contrast, increased serum cholesterol was observed in male PND 90 offspring following exposure to 700 mg/kg/day from GD 13 to PND 21 (Venturelli et al. 2019). Serum triglycerides and cholesterol levels were not observed when young Wistar rats were similarly exposed for 30 days post-weaning (Venturelli et al. 2015). No change in liver weight was observed following gestational plus lactational, lactational, or post-weaning exposure paradigms; histology was not assessed.

Age-dependent effects on enzyme activities were examined in rats of three ages: 3, 6, and 10 weeks old (Parmar et al. 1994). Single administration of 2,000 mg DEHP/kg decreased the cytochrome P-450 contents in the liver, as well as the activities of aryl hydrocarbon hydroxylase (AHH), aniline hydroxylase, and ethylmorphine N-demethylase in all age groups, while repeated exposure induced them

with maximum increases occurring in 3-week-old rats. Administration of DEHP for 15 days decreased cytochrome P-450 and the activity of the three enzymes only in the 3-week-old rats. Six- and 10-week-old rats showed an inhibition of AHH and increased activities of aniline hydroxylase and ethylmorphine N-demethylase, which were lower than seen after 7 days of exposure in their respective groups. The potential adversity of observed changes in the MFO enzymes on the liver is difficult to determine in the absence of evaluation of other hepatic endpoints. Changes could potentially lead to altered metabolism of endogenous and exogenous chemicals, resulting in decreased detoxification of chemicals and/or decreased formation of toxic intermediates.

Animal Studies—Renal System Development. In the only inhalation study evaluating potential effects on the developing renal system following DEHP exposure, no changes in kidney weights were observed in female weanling rats exposed to DEHP at concentrations up to 1.6 ppm for 6 hours/day, 5 days/week for 3 or 9 weeks (Ma et al. 2006). No other renal parameters were measured.

In orally exposed nonhuman primates, no changes in clinical chemistry measures of renal function, urinalysis parameters, or kidney weight or histology were observed in 14-day studies in sexually immature Cynomolgus monkeys at 500 mg/kg/day (Pugh et al. 2000).

In a developmental study in Wistar rats, impaired kidney development and function were observed in adult offspring following maternal exposure to 0.25 or 6.25 mg/kg/day from GD 0 to PND 21 (Wei et al. 2012). Creatinine clearance (measured at PNW 21) was significantly reduced in all exposed offspring. Serum creatinine was only significantly elevated in low-dose female offspring. Serum BUN was significantly elevated in low-dose females and low- and high-dose males, and urinary total protein was significantly elevated in low- and high-dose females and high-dose males. Serum renin and angiotensin levels were reduced at birth but increased at PNW 3. The glomerular number per kidney was significantly decreased (compared with control) at PNWs 3 and 33 in all exposed offspring; total glomerular volume was also decreased at PNW 33 in all exposed offspring. The average individual glomerular volume was increased in high-dose females and all exposed males at PNW 3 but decreased in all exposed males at PNW 33.

The Wei et al. (2012) study that reported impaired kidney development and function also showed decreased glomerular size, glomerular swelling, and reduction in Bowman’s capsule size in both exposure groups from PND 0 to PNW 33. Electron microscopy showed renal tubular dilation, tubular atrophy,

interstitial fibrosis, and scarring. Additionally, significant increases in blood pressure in exposed offspring were considered secondary to impaired kidney function.

Wei et al. (2012) also observed significant changes in offspring kidney weights, some of which may be attributable to observed decreases in offspring body weight. High-dose females at PNW 15 had significantly decreased absolute kidney weight and body weight. Females in the low-dose group at PNW 15 had slightly decreased absolute kidney weight and slightly increased body weight, resulting in a significantly decreased relative kidney weight. High-dose males at PNWs 15 and 21 had increased absolute kidney weight and decreased body weight. At birth and weaning (PNW 3), pups had significant decreases in body weight with minimal decreases in kidney weight, resulting in statistically significant relative kidney weights on these days.

Reversible decreases in kidney weights (recovered by PND 56), reversible glomerulonephritis and dilation of renal tubule (recovered by PND 42), and persistent light renal fibrosis (no recovery at PND 56) were seen in offspring of Long-Evans rat dams exposed to DEHP at ≥3 mg/kg/day during all of gestation and lactation (Arcadi et al. 1998). In Sprague-Dawley rat offspring, decreased kidney weight was observed in adulthood following gestation and lactational exposure to a maternal dose of 300 mg/kg/day, but not at maternal doses up to 100 mg/kg/day (Gray et al. 2009). A subset of male offspring continued direct exposure post-weaning through PND 65; decreased kidney weight was also observed at 300 mg/kg/day in these animals. However, no exposure-related changes in kidney weights were observed in neonatal, weanling, or adult offspring born to Sprague-Dawley rat dams exposed to DEHP at doses up to 405 mg/kg/day during gestation and lactation (Andrade et al. 2006a; Grande et al. 2007; Kobayashi et al. 2006; Nardelli et al. 2017).

In a 2-generation study in Wistar rats, absolute kidney weights were decreased in F2 weanlings exposed to 1,088 mg/kg/day, but relative kidney weights were increased at lower doses (113 and 340 mg/kg/day); no exposure-related changes were observed in kidney weights in F1 weanlings (Schilling et al. 2001). No exposure-related changes were observed in Wistar rat offspring on PND 16 following maternal exposure to doses up to 900 mg/kg/day from GD 7 to PND 16 (Christiansen et al. 2010), or in adult offspring following maternal exposure to doses up to 700 mg/kg/day from GD 13 to PND 21 or 75 mg/kg/day from PND 1 to 21 (Venturelli et al. 2015, 2019). Similarly, no changes in kidney weight were observed in young Wistar rats exposed to doses up to 75 mg/kg/day for 30 days post-weaning (Venturelli et al. 2015). Measures of renal function and kidney histology were not assessed in these studies. No changes in kidney weight or histology were observed in male PND 7 Sprague-Dawley rats following direct exposure

on PNDs 3–7 to doses up to 600 mg/kg/day (Camacho et al. 2020). However, with longer exposure (PNDs 3–23), decreased kidney weight and renal tubule degeneration were observed at ≥300 mg/kg/day.

Significant increases in serum urea and creatinine were observed in male PND 80 Wistar rat offspring following maternal exposure to DEHP at doses ≥10 mg/kg/day from GD 9 to PND 21 (Rajagopal et al. 2019a). Kidney weight and histology were not assessed.

Epidemiology Studies—Neurodevelopment. Many epidemiological studies assessed neuro-developmental outcomes. The types of neurodevelopmental effects that have been evaluated include infant neurological state; cognitive, mental and psychomotor development; behavior and emotional development; social development and autism spectrum disorders; and gender-related behaviors. All of the selected studies (Table 2-14) are birth cohort studies that evaluated exposure using maternal urine samples; some studies additionally evaluated child urine samples.

In a study using the neonatal intensive care unit (NICU) Network Neurobehavioral Scale (NNNS) to evaluate infant neurological state, Yolton et al. (2011) observed an association between increased frequency of nonoptimal reflexes in male infants (n=158 boys) and the sum of DEHP metabolites in maternal urine samples collected at 26±4 weeks of gestation (β =0.216, SE=0.090, p=0.02). No association was seen between female infants (n=174 girls) and DEHP metabolites in maternal urine samples collected at 26 weeks, or in either sex using maternal urine samples collected at 16 weeks. No other subscales of the NNNS (e.g., attention, arousal, regulation, handling, etc.) were affected in boys or girls.

The database for epidemiological studies of cognitive/mental and psychomotor development includes 26 studies of 13 birth cohorts (Table 2-14). Many cohorts were longitudinal in design, evaluating cognitive/mental and psychomotor development across several ages. These studies used standard instruments for assessing development; typically, the Bayley Score for Infant Development (BSID) was used in children up to 3 years of age and the Wechsler Intelligence Scale for Children (WISC) was used in older children. However, the available studies measuring these endpoints are not strictly comparable, due to differences in the instruments used to assess development, varying ages at assessment, gestational timing of maternal urine collection, nature and number of covariates considered in the analyses, differences in study populations, and specific DEHP metabolites measured in urine. Of the selected studies, three suggested associations between poorer performance on the mental development index at 6 months (Kim et al. 2011), 23–26 months of age (Qian et al. 2019a, 2019b), and 2–3 years of age

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Columbia Center for Children's Environmental Health (CCCEH) cohort studies				
Ipapo et al. 2017 168 children, FTII administered at 27 weeks of age, United States (New York)	Visual recognition memory	ΣDEHP	NR	↔
		MEHP	Maternal (3rd trimester) IQR: 1.90–9.85 ng/mL	↔
		MEHHP	10.25–40.80	↔
		MEOHP	8.35–37.10	↔
		MECPP	17.95–74.55	↔
Whyatt et al. 2012 319 children (151 boys, 168 girls), BSID-II (MDI and PDI) administered to infants between 27 and 42 months of age (mean 36.4 months), CBCL when children were between 33 and 48 months of age (mean 36.6 months), United States (New York)	BSID or CBCL	ΣDEHP	NR	↔
		MEHP	Maternal (3rd trimester) range: <LOD–613 ng/mL	NR
		MEHHP	1.1–1,750	NR
		MEOHP	0.7–1,320	NR
		MECPP	3.0–1,840	NR
Factor-Litvak et al. 2014 328 children (155 boys, 173 girls), WISC administered to children at 7 years of age, United States (New York)	WISC	MEHP	Maternal (3rd trimester) IQR: 1.9–12.4 ng/mL	↔
		MEHHP	10.6–47.2	↔

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Balalian et al. 2019; Daniel et al. 2020 209 children (93 boys, 116 girls, BOT-2 administered at 11 years of age, United States (New York)	**Total motor skills**	**ΣDEHP** (MEHP, MEHHP, MEOHP, MECPP)	Maternal (3rd trimester) IQR: 137.1–577.7 (molar sum)	↔
			Child (age 3): 186.80–919.3	↔
			Child (age 5): 203.53–852.9	↔
			Child (age 7): 245.7–865.4	↓ **(Boys)** ↔ (Girls)
	Fine motor skills	**ΣDEHP**	Maternal, child (age 5): see above	↔
			Child (age 3 or 7): see above	↓ **(Boys)** ↔ (Girls)
	Gross motor skills	ΣDEHP	Maternal or child (any age)	↔
Health Outcomes and Measures of the Environment (HOME) cohort studies				
Yolton et al. 2011 350 infants (163 boys, 187 girls), NNNS measured at 5 weeks of age, United States (Ohio)	**Nonoptimal reflexes**	**ΣDEHP** (MEHP, MEHHP, MEOHP, MECPP)	Maternal (16 weeks) GM (95% CI): 311 (269–360) nmol/L	Boys: ↔ Girls: ↔
			Maternal (26 weeks): 245 (213–281)	**Boys**: ↑ Girls: ↔
		No significant association for other subscales of the NNNS in males or females.		
Braun et al. 2014 175 children, SRS administered at 4–6 years of age, United States (Ohio)	SRS	MEHP	Maternal IQR (average of 16 and 26 weeks): 2.9–7.5 µg/g Cr	↔
		MEHHP	15–49	↔
		MECPP	21–70	↔
Braun et al. 2017a, 2017b 198 children (91 boys, 107 girls), VMWM administered at 8 years of age, United States (Ohio)	Visual-spatial abilities	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	Maternal (average of 16 and 26 weeks) IQR: 44–148 µg/g Cr	All: ↔ Boys: ↔ Girls: ↔

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Percy et al. 2016 227 children (101 boys, 126 girls), mothers completed GIQ and children completed PPPSI at 8 years of age, United States (Ohio)	GIQ or PPPSI	ΣDEHP	Maternal (16 weeks) GM (95% CI): 87.9 (73.4, 105.2) nmol/L Maternal (26 weeks): 65.9 (55.2, 78.5)	↔
		MEHP	Maternal (16 weeks) GM (95% CI): 4.9 (4.1, 6) ng/mL Maternal (26 weeks): 4.3 (3.6, 5)	↔
		MEHHP	Maternal (16 weeks): 27 (22.4, 32.7) Maternal (26 weeks): 19.4 (16.1, 23.4)	↔
		MEOHP	Maternal (16 weeks): 20.1 (16.7, 24.2) Maternal (26 weeks): 15.9 (13.2, 19.2)	↔
		MECPP	Maternal (16 weeks): 39.3 (33, 46.9) Maternal (26 weeks): 29.1 (24.5, 34.6)	↔
Mount Sinai Children's Environmental Health Study cohort				
Doherty et al. 2017 250 children (134 boys, 116 girls), BSID (MDI and PDI) administered at approximately 24 months of age, United States (New York)	MDI and PDI	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	Maternal (31 weeks) GM (SE): 0.28 (3.7) µmol/L	↔
Miodovnik et al. 2011 137 children, SRS administered at 7–9 years of age, United States (New York)	SRS	ΣDEHP ((MEHP, MEHHP, MEOHP, MECPP)	NR	↔

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Study for Future Families (SFF) cohort studies				
Swan et al. 2010 145 children (74 boys, 71 girls), mothers completed PSAI when children were approximately 5 years old, United States (California, Minnesota, Missouri, Iowa)	**PSAI scores for masculine play**	**ΣDEHP**	Maternal (mid-pregnancy) IQR: 11.7, 40.3 ng/mL	**Boys: ↓** Girls: ↔
		MEHP	1.4, 6.2	Boys: ↔ Girls: ↔
		MEHHP	5.2, 17.3	**Boys: ↓** Girls: ↔
		MEOHP	4.7, 17.9	**Boys: ↓** Girls: ↔
	PSAI scores for composite or feminine play	ΣDEHP, MEHP, MEHHP, MEOHP	See above	↔
Kobrosly et al. 2014 153 children (77 boys, 76 girls), mothers completed CBCL when children were 72–126 months of age (mean 102 months or 8.5 years), United States (California, Minnesota, Missouri, Iowa)	**Anxiety/depression**	**ΣDEHP**	NR	All: ↔ Boys: ↔ **Girls**: ↓
		MEHP	Maternal (26 weeks) IQR: 1.1, 9.9 ng/mL	NR
		MEHHP	6.1, 24.2	NR
		MEOHP	5.1, 22.0	NR
		DEHP metabolites were not associated with other CBCL behavioral scores.		
Taiwan maternal and infant cohort studies				
Huang et al. 2015 110 children (58 boys, 52 girls), BSID-II administered at age 2 years; WPPSI-R at age 5 years; WISC-III at age 8 years, and WISC-IV at age 11 years, Taiwan	IQ	MEHP	Maternal (3rd trimester) GM (95% CI): 19.79 (16.38, 23.92) µg/g Cr	↔
		MEHHP	8.49 (5.97, 12.09)	↔
		MEOHP	12.97(9.23, 18.21)	↔
		Decreased IQ was associated with increased MEOHP and ∑DEHP metabolites in child's urine; however, samples were taken at the same time as tests administered.		

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Ku et al. 2020 208 children, CTTS administered at age 2 years, BSQ-C at age 5 years, and MCTQ-C at age 11 years, Taiwan	**Withdrawal**	**ΣDEHP**	NR	**2 years:** ↑ 5/11 years: ↔
		MEHP	Maternal (3rd trimester) GM (95% CI): 19.20 (16.69, 22.09) µg/g Cr	**2 years:** ↑ 5/11 years: ↔
		MEHHP	8.24 (6.39,10.62)	**2 years:** ↑ **5 years:** ↓ 11 years: ↔
		MEOHP	12.41 (9.79,15.73)	**2 years:** ↑ **5 years:** ↓ 11 years: ↔
	Threshold of responsiveness	**ΣDEHP, MEHP, MEOHP**	See above	**2/5 years:** ↓ 11 year: ↔
		MEHHP	See above	↔
	Distractibility	**ΣDEHP, MEHP**	See above	**2 years:** ↑ 5/11 year: ↔
		MEHHP, MEOHP	See above	↔
	Intensity of reaction	**ΣDEHP, MEHP**	See above	**2 years:** ↓ 5/11 years: ↔
		MEHHP, MEHOP	See above	↔
	Activity level	ΣDEHP, MEHHP, MEHOP	See above	↔
		MEHP	See above	2/5 years: ↔ **11 years:** ↑
		DEHP metabolites in maternal urine were not associated with other temperament scores. DEHP metabolites in child's urine were associated with increased adaptability and decreased persistence at 2 years of age, decreased positive mood and withdrawal at 5 years of age, and decreased intensity of reaction and increased odds of ADHD symptoms at 11 years of age; however, samples were taken at the same time as tests administered.		

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Lien et al. 2015 122 children, mothers completed CBCL when children were 8 years of age, Taiwan	**Delinquent behavior (clinical range)**	MEHP	Maternal (3rd trimester) GM (95% CI): 16.93 (14.32, 20.02) µg/g Cr	↔
		MEHHP	7.91 (5.69, 11.02)	↔
		MEOHP	13.59 (10.27, 18.00)	↑
	Aggressive behavior, externalizing problems (clinical range), or borderline or borderline/clinical scores for all behaviors	**MEHP, MEHHP, MEOHP**	See above	↑
Chen et al. 2019 122 children, mothers completed CBCL when children were 8, 11, and 14 years of age (results combined in analysis), Taiwan	**Delinquent behavior, externalizing problems**	**ΣDEHP**	NR	**All:** ↑ **Boys:** ↑ **Girls:** ↑
		MEHP	Maternal (3rd trimester) GM (range): 16.93 (1.79, 706.10) µg/g Cr	NR
		MEHPP	7.91 (0.05, 489.28)	NR
		MEOHP	13.59 (0.16, 1010.72)	NR
	Withdrawn, social problems, internalizing problems	**ΣDEHP**	NR	**All:** ↑ **Boys:** ↑ Girls: ↔
	Aggressive behavior	**ΣDEHP**	NR	**All:** ↑ Boys: ↔ **Girls:** ↑
	Anxious/depressed, thought problems, attention problems	**ΣDEHP**	NR	**All:** ↑ Boys: ↔ Girls: ↔
	Somatic complaints	ΣDEHP	NR	↔

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Huang et al. 2019 153 children, mothers completed CBCL when children were 8, 11, and 14 years of age (results combined in analysis), Taiwan	**Anxious/depressed, social problems, thought problems, attention problems, aggressive behavior, internalizing problems**	ΣDEHP (MEHP, MEHHP, MEOHP)	Maternal (3rd trimester) GM (95% CI): 0.17 (0.15, 0.20) µmol/g Cr	↔
		MEHP	16.73 (14.46, 19.36) µg/g Cr	↑
	Delinquent behavior, externalizing problems; borderline or clinical internalizing or externalizing problems	**ΣDEHP, MEHP**	See above	↑
		DEHP metabolites in maternal urine were not associated with other CBCL behavioral scores. DEHP metabolites in child's urine at 2–3, 5–6, or 8–9 years of age were not associated with any CBCL behavioral scores at 8–14 years of age.		
Other cohort studies				
Engel et al. 2018 MoBa cohort (nested case-control), 850 children including 297 with ADHD and 553 without ADHD evaluated at ≥3 years (cases and controls combined for analysis), Norway,	**ADHD**	**ΣDEHP** (MEHP, MEHHP, MEOHP, MECPP)	Maternal (17 weeks) IQR: Case: 0.21–0.41 µmol/L Control: 0.18–0.34	**All:** ↑ **Boys:** ↑ Girls: ↔
Gascon et al. 2015b INMA cohort, 367 children (187 boys, 178 girls), BSID-II (MDI and PDI) administered at 1 year of age, MSCA, CPSCS and ADHD evaluated at age 4 years, SDQ and short form of CSRS (includes ADHD index) evaluated at age 7 years, Spain	BSID-II or MSCA	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	Maternal (average 1st and 3rd trimester) IQR: 68–146 µg/g Cr	↔
	Social competence (4 years)	**ΣDEHP**	See above	↑
	Risk of inattention symptoms, ADHD (4 and 7 years)	**ΣDEHP**	See above	↓

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Kim et al. 2018f CHECK cohort, 86 children, BSID-II (MDI and PDI), SMS (social quotient), and CBCL were administered at 13–24 months of age, Republic of Korea	BSID-II, SMS, or CBCL	MEHP	Maternal (delivery) IQR: 7.8–19.1 µg/g Cr	↔
		MEHHP	14.9–35.4	↔
		MEOHP	13.5–31.1	↔
Kim et al. 2011 MOCEH cohort, 460 children (235 boys and 225 girls), BSID-II (MDI and PDI) administered to infants at 6 months of age, Republic of Korea	**MDI and PDI**	**MEHHP**	Maternal (3rd trimester) IQR: 4.3–21.4 ng/mL	**Boys: ↓** Girls: ↔
		MEOHP	3.8–17.1	**Boys: ↓** Girls: ↔
Olesen et al. 2018a, 2018b OCC cohort, 518 children (271 boys, 247 girls), MB-CDI administered every third month from 16 to 36 months of age, Demark	**MB-CDI (vocabulary or complexity score)**	**ΣDEHP**	Maternal (week 28) IQR: 10.0–34.4 ng/mL	**Boys: ↓** Girls: ↔
		MEHP	0.5–2.2	Boys: ↔ Girls: ↔
		MEHHP	2.2–8.6	**Boys: ↓** Girls: ↔
		MEOHP	2.0–6.9	**Boys: ↓** Girls: ↔
		MECPP	2.5–8.5	**Boys: ↓** Girls: ↔

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
Polanska et al. 2014 REPRO_PL Cohort, 165 children (72 boys, 93 girls), BSID-III administered to infants at 24 months of age, Poland	**Motor scores**	**ΣDEHP**	Maternal (3rd trimester) range: 0.0004–1.5 µmol/g Cr	↓
		MEHP	0.02–4.3 µg/g Cr	↔
		MEHHP	0.02–431	↓
		MEOHP	0.04–140	↓
	MDI: cognitive scores; language scores	ΣDEHP, MEHP, MEHHP, MEOHP	See above	↓
Qian et al. 2019a, 2019b Wuhan prenatal cohort, 476 children, BSID-CR (MDI and PDI) administered at 23–26 months of age, China	**MDI**	**ΣDEHP**	Maternal (average across three trimesters) median: 91.71 nmol/L	↔
			1st trimester: 81.14	↔
			2nd trimester: 70.35	↔
			3rd trimester: 88.22	**All:** ↓ Boys: ↔ Girls: ↔
		MEHP	Maternal (average) median: 3.23 µg/L	↔
			1st trimester: 2.80	↔
			2nd trimester: 2.26	↔
			3rd trimester: 2.41	↔
		MEHHP	Maternal (average): 6.91	↔
			1st trimester: 6.31	↔
			2nd trimester: 4.89	↔
			3rd trimester: 6.48	**All:** ↓ **Boys:** ↓ Girls: ↔

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
		MEOHP	Maternal (average): 5.62	↔
			1st trimester: 4.81	↔
			2nd trimester: 4.18	↔
			3rd trimester: 5.38	↔
		MECPP	Maternal (average): 10.62	↔
			1st trimester: 9.31	↔
			2nd trimester: 8.14	↔
			3rd trimester: 10.54	**All:** ↓ **Boys:** ↓ Girls: ↔
	PDI	**ΣDEHP**	Maternal (average or 3rd trimester): see above	All: ↔ **Boys:** ↑ Girls: ↔
			1st or 2nd trimester: see above	↔
		MEHP	Maternal (average or 1st trimester): see above	**All:** ↔ **Boys:** ↑ Girls: ↔
			2nd trimester: see above	All: ↔ **Boys:** ↑ Girls: ↔
			3rd trimester: see above	↔

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
		MEHHP	Maternal (average, 1st, 2nd, or 3rd trimester): see above	↔
		MEOHP	Maternal (average or 3rd trimester): see above	All: ↔ **Boys**: ↑ Girls: ↔
			1st or 2nd trimester: see above	↔
		MECPP	Maternal (average): 10.62	**All:** ↑ **Boys:** ↑ Girls: ↔
			1st or 2nd trimester: see above	↔
			Maternal (3rd trimester): 10.54	All: ↔ **Boys:** ↑ Girls: ↔
Téllez-Rojo et al. 2013 ELEMENT cohort, 135 children (64 boys, 71 girls), BSID-II (MDI and PDI) administered to children at 24, 30, and 36 months of age (results combined in analysis), Mexico	**MDI**	**ΣDEHP**	Maternal (3rd trimester) GM (95% CI): 0.35 (0.30, 0.40) nmol/mL (SG-adj)	All: ↔ Boys: ↔ **Girls:** ↓
		MEHP	6.56 (5.72, 7.53) ng/mL (SG-adj)	All: ↔ Boys: ↔ **Girls:** ↓
		MEHHP	22.08 (18.77, 25.96)	All: ↔ Boys: ↔ **Girls:** ↓
		MEOHP	14.23 (12.05, 16.80)	All: ↔ Boys: ↔ **Girls:** ↓

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference, population	Outcome evaluated	Metabolite	Urine concentration[a]	Result
		MECPP	39.65 (34.32, 45.81)	All: ↔ Boys: ↔ **Girls: ↓**
		DEHP metabolites were not associated with PDI scores in either sex.		

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; ADHD = attention-deficit/hyperactivity disorder; BOT-2 = Bruininks-Oseretsky Test of Motor Proficiency-2; BSID = Bayley Scales of Infant Development; BSID-CR = Bayley Scales of Infant Development Chinese Revision; BSQ-C = Behavior Style Questionnaire-Chinese version; CBLC = child behavior checklist; CHECK = Children's Health and Environmental Chemicals in Korea cohort; CI = confidence interval; CPSCS = California Preschool Social Competence Scale; Cr = creatinine; CSRS = Connors' Parent Rating Scales; CTTS = Chinese Toddler Temperament Scale; DEHP = di(2-ethylhexyl)phthalate; ELEMENT = Early Life Exposure in Mexico to Environmental Toxicants; FTII = Fagan Test of Infant Intelligence; GIQ = Gender Identity Questionnaire; GM = geometric mean; INMA = Infancia y Medio Ambiente (Environment and Childhood) birth cohort; IQ = intelligence quotient; IQR = interquartile range; LOD = limit of detection; MCTQ-C = Middle Childhood Temperament Questionnaire-Chinese version; MDI = Mental Development Index; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; MoBa = Norwegian Mother and Child Cohort; MOCEH = Prospective Mothers and Children's Environmental Health Cohort; MSCA = McCarthy Scales of Children's Abilities; NNNS = NICU Network Neurobehavioral Scale; OCC = Odense Child Cohort; PDI = Psychomotor Development Index; PPPSI = Playmate and Play Style Preferences Structured Interview; PSAI = preschool Activities Inventory; REPRO_PL = Polish Mother and Child Cohort; SDQ = Strengths and Difficulties Questionnaire; SG-adj = specific gravity adjusted; SMS = social maturity scale; SRS = Social Responsiveness Scale; VMWM = Virtual Morris Water Maze; WISC = Wechsler Intelligence Scale for Children; WWPSI-R = Wechsler Preschool and Primary Scale of Intelligence-Revised

(Téllez-Rojo et al. 2013) and prenatal DEHP exposure. The affected sex differed between the studies with Kim et al. (2011) and Qian et al. (2019a, 2019b) reporting an association for male infants and Téllez-Rojo et al. (2013) observing an association only in female infants. A fourth study reported impaired language development in boys, but not girls, between 16 and 36 months of age with increased prenatal DEHP exposure (Olesen et al. 2018a, 2018b). Three studies (Kim et al. 2011; Polanska et al. 2014; Qian et al. 2019a, 2019b) reported associations between prenatal DEHP exposure and psychomotor development index in young children (6–26 months); two of the studies (Kim et al. 2011; Qian et al. 2019a, 2019b) observed the association in males only. Using a different assessment (BOT-2; Bruininks-Oseretsky Test of Motor Proficiency-2), no association between prenatal DEHP exposure and motor skills at age 11 was observed (Balalian et al. 2019; Daniel et al. 2020). However, motor skills at 11 years of age, particularly fine motor skills, were impaired in boys only with increased DEHP metabolites in children's urine collected at 3 or 7 years (Balalian et al. 2019; Daniel et al. 2020). Other birth cohort studies did not observe an association between maternal urinary DEHP metabolite levels and cognitive/mental or psychomotor abilities children assessed at ages ranging from 6 months to 11 years of age (Braun et al. 2017a, 2017b; Doherty et al. 2017; Factor-Litvak et al. 2014; Gascon et al. 2015b; Huang et al. 2015; Ipapo et al. 2017; Kim et al. 2018f; Whyatt et al. 2012).

The database for epidemiological studies of behavior and attention includes 13 studies of 9 birth cohorts (Table 2-14). Evaluations included various validated measures of overall behavioral development, social behavior (including screening for social impairments related to Autism Spectrum Disorder [ASD]), gender-related play, and measures of attentiveness (including screening for Attention Deficit Hyperactivity Disorder [ADHD]). In these cohorts, increases and decreases in a variety of behaviors have been associated with increased prenatal DEHP exposure; however, comparison across studies is complicated due to differences in the instruments used to assess development, varying ages at assessment, gestational timing of maternal urine collection, nature and number of covariates considered in the analyses, differences in study populations, and specific DEHP metabolites measured in urine. Social Responsiveness Scale (SRS), which is a validated scale for measuring ASD-related behaviors, no association between social impairment and prenatal DEHP exposure was observed in children at ages 4–6 years (Braun et al. 2014) or 7–9 years (Miodovnik et al. 2011). Another study reported improved social competence in 4-year-olds with increasing prenatal DEHP exposure (Gascon et al. 2015a). Other studies examining potential relationships between DEHP exposure and ASD are limited to case-control studies in which exposure was measured after the diagnosis (Kardas et al. 2016; Stein et al. 2013; Testa et al. 2012); these studies were not included in Table 2-14 or considered useful for hazard identification.

Two cohort studies evaluated potential associations between gender-related play in children and maternal urinary DEHP metabolite levels (Percy et al. 2016; Swan et al. 2010). In a multicenter U.S. birth cohort (74 boys, 71 girls), prenatal maternal urinary metabolite levels were associated with reduced scores on the Pre-School Activities Inventory (PSAI), indicative of decreased masculine play activities, among 5-year-old boys (Swan et al. 2010). In contrast, another the U.S. birth cohort (101 boys, 126 girls) did not observe associations between maternal urinary metabolite levels and scores on the Gender Identity Questionnaire (GIQ) and the Playmate and Play Style Preferences Structured Interview (PPSI) measures of gender-related play at 8 years of age (Percy et al. 2016). Results from these studies are difficult to compare, primarily due to use of different metrics and different ages at analysis.

In 3–12-year-old children recruited in Taiwan after the phthalate-tainted food scandal in 2011, current urinary MEOHP levels were inversely associated with verbal comprehension in school-aged children; no association between current DEHP urinary levels and cognitive/mental and psychomotor development were observed in preschoolers (Huang et al. 2017). When past DEHP exposure was estimated (prior to 2011), no association with past exposure was observed.

Animal Studies—Neurodevelopment. One inhalation developmental study in Wistar rats evaluated neurodevelopment in the offspring of females exposed to up to 21 ppm for 6 hours/day from GD 6 to 15 (Merkle et al. 1988). Newborn rats did not show any evidence of altered neurological development in the righting test on PND 6, gripping reflex on PND 13, pupillary reflex on PND 20, or hearing test on PND 21.

In oral developmental studies, neurobehavioral changes have been observed following gestational or gestational plus lactational exposure to DEHP. Impaired performance on the learned avoidance test was observed in PND 30 female offspring of Long-Evans rat dams exposed to 30 mg/kg/day during gestation and lactation; this was not observed in female offspring after maternal exposure to 3 mg/kg/day or in male offspring after maternal doses up to 30 mg/kg/day (Arcadi et al. 1998). The study authors reported that it was unclear whether the observed neurobehavioral effects were due to learning and memory deficits, muscle weakness, impaired motor coordination (particularly of the hindlimbs), or alterations in motivation (fear) and attentional components. Locomotor activity measured during both light and dark cycles was significantly decreased by up to 40% in adult offspring of Sprague-Dawley rat dams exposed to 300 mg/kg/day from GD 14 to PND 0 (only dose tested) (Martinez-Arguelles et al. 2013). No other measures of neurobehavior were conducted.

Increased anxiety in an open field was shown in CD-1 mouse offspring at 18 months of age following maternal exposure to ≥0.2 mg/kg/day from GD 11 until parturition (Barakat et al. 2018). However, the anxiety effects observed at the low dose are difficult to interpret because another measure of anxiety (elevated plus maze) did not show significant increases in anxiety in 18-month-old offspring until maternal doses of 750 mg/kg/day. At 16 months of age, mouse offspring also showed impaired recognition memory at maternal doses ≥500 mg/kg/day and impaired spatial memory at the maternal dose of 0.2 mg/kg/day (but not ≥500 mg/kg/day). No evidence of overall changes in activity levels were observed during these behavioral assessments (Barakat et al. 2018). Consistent with this study, no changes in object recognition and/or spontaneous locomotion were observed in mouse offspring exposed to a maternal dose of 33 mg/kg/day during gestation and lactation (Bastos Sales et al. 2018) or up to 95 mg/kg/day during gestation only (NTP 1988).

Altered behavior has also been reported at 30 mg/kg/day following early postnatal exposure. In a series of experiments that evaluated anxiety-like behavior in Wistar rats using the elevated plus maze, male rats exposed to 30 mg/kg/day from PND 1 to 21 (via lactation) plus PNDs 22–45 or 22–60 (via drinking water) showed increased anxiety-like behavior (Carbone et al. 2013). Observed effects included fewer entries into the open and closed arms, less time in the open arms, and more time in the closed arms. No behavioral changes were observed in similarly exposed females. When direct exposure ceased at PND 30, altered behavior in the elevated plus maze was not observed in either sex (Carbone et al. 2013).

In a 2-generation study in Wistar rats evaluating doses up to approximately 1,088 mg/kg/day, F2 offspring were evaluated for neurological effects using FOB on PND 28 and water maze testing (for learning and memory) on PNDs 28 and 35 (Schilling et al. 2001). The only changes observed in the FOB were decreased grip strength and foot splay in high-dose animals; however, these effects were attributed to decreased body weights observed at this dose. No exposure-related changes were observed in the water maze. However, in a 1-generation study in CD-1 mice (4 weeks premating through PNW 9), a delayed surface righting reflex was observed at PND 4 and 7 in female F1 offspring at ≥20.62 mg/kg/day (lowest dose tested) and at PND 7 in male F1 offspring at ≥60.42 mg/kg/day (Tanaka 2002). No exposure-related changes were observed in negative geotaxis on PNDs 4 and 7, cliff avoidance on PND 7, swimming behavior on PNDs 4 and 14, olfactory orientation on PND 14, exploratory behavior on PNDs 21 and 56, or learning and memory in a multiple water T-maze on PND 49 at doses up to 180.77 mg/kg/day (Tanaka 2002).

Brain weights and the numbers of dopaminergic neurons were evaluated at PNWs 2, 4, and 6 in ICR mice exposed to 0 or 1 mg/kg/day from GD 8 to 17 (via dams) and PNDs 3–7 (direct exposure) (Tanida et al. 2009). Significant changes included 4 and 8% decreases in absolute and relative brain weights at PNW 6, respectively, and a 15% decrease in relative brain weight at 2 weeks. The numbers of tyrosine hydroxylase- and Fos-immunoreactive neurons were significantly decreased at PNWs 4 and 6, indicating a decrease in dopaminergic neurons (tyrosine hydroxylase is a marker for biosynthetic activity of dopamine; Fos is a marker of neuronal activation). No changes in brain weight were observed in CD-1 mouse offspring at 22 months of age following maternal exposure to doses up to 750 mg/kg/day from GD 11 until parturition (Barakat et al. 2018). However, a significant decrease in the number of pyramidal neurons in the hippocampus was observed at maternal doses ≥0.2 mg/kg/day, and neurons were shrunken and loosely aligned with enlarged inter-neural space between neurons.

In nonhuman primates, no changes in brain weight occurred in sexually immature Cynomolgus monkeys exposed to 500 mg/kg/day for 14 days (Pugh et al. 2000). In Sprague-Dawley rats, no exposure-related changes in brain weights were observed at PND 1 or 21 in offspring following maternal doses up to 405 mg/kg/day from GD 6 to PND 21 (Andrade et al. 2006c; Grande et al. 2006). Similarly, no exposure-related changes were observed in F1 or F2 pup brain weight in a 2-generation study in Sprague-Dawley rats at doses up to 1,088 mg/kg/day (Schilling et al. 2001).

Mechanisms of Neurodevelopmental Toxicity. Several animal studies indicate that DEHP alters hippocampal structural and functional plasticity following pre-, peri-, and post-natal exposure. Sun et al. (2014a) reported evidence of altered hippocampal function (impaired memory and learning) and impaired structural plasticity (elevated levels of phosphorylated Tau with no increase in total Tau) in adult rat offspring following perinatal exposure to DEHP. Tau hyperphosporylation of microtubule proteins was associated with reduced neurite outgrowth in mouse neuroblastoma cells *in vitro* (Wang et al. 2017b). In mice, impaired functional plasticity was suggested by inhibition of ERK1/2 phosphorylation in the hippocampus following perinatal DEHP exposure (Xu et al. 2015). Structural changes in the hippocampus have also been observed in juvenile and adult rats following pre- or postnatal exposure to DEHP, including decreased axonal innervation, decreased cell density, decreased dendritic spine density, decreased dendritic length and branching, and reduced neurogenesis (Barakat et al. 2018; Smith and Holahan 2014; Smith et al. 2011; You et al. 2018). Structural changes in hippocampal pyramidal neurons following developmental exposure to DEHP were associated with decreases in microtubule-associated protein 2 (MAPc2) and stathmin, which are both key molecules for neural cytoskeleton synthesis (You et

al. 2018). Neuronal excitability and synaptic plasticity were also reduced in rat hippocampal slices incubated with DEHP via inhibition of the voltage-gated potassium channel (Ran et al. 2019).

Disruption of calcium homeostasis may contribute to DEHP-mediated neurotoxicity. Neuronal degeneration has been associated with increased intracellular calcium levels, resulting in inhibition of cellular membrane Na+/K+-ATPase activity, in rats following intraperitoneal exposure to DEHP (Dhanya et al. 2003). DEHP also increased intracellular calcium levels in rat neurohypophysial nerve terminals and pheochromocytoma cells (Tully et al. 2000). Additionally, DEHP decreased calcium signaling mediated through the nicotinic acetylcholine receptor in human neuroblastoma cells (Kaun-Yu et al. 2004).

As discussed extensively in Section 2.9 (Hepatic), DEHP activation of PPARs is a key mechanistic event for hepatic toxicity (Kushman et al. 2013; Rusyn and Corton 2012). Neurodevelopmental toxicity may also be mediated by PPAR activation. In support, Lin et al. (2011) indicated that PPARγ overexpression induced by DEHP may result in apoptosis of undifferentiated neurons. PPAR activation may also contribute to observed changes in fetal lipid metabolome, including reduction in the overall lipid content and alterations in fatty acid composition of the fetal rat brain observed following exposure to DEHP during gestation (Xu et al. 2007, 2008).

Neurodevelopmental effects of DEHP may be related to decreased thyroid hormone transfer across the placenta. DEHP was shown to disrupt thyroid hormone uptake in placental trophoblastic cells through mechanisms involving reduced expression and internalization of transthyretin (Du et al. 2020).

Observed DEHP-moderated alterations in oxidative stress and inflammatory pathways (Barakat et al. 2018; Ferguson et al. 2012, 2015, 2017; Wu et al. 2017) could potentially contribute to neurodevelopmental toxicity of DEHP. DEHP induced oxidative stress in cultured mouse neural stem cells, which was associated with cytotoxicity and apoptosis (Wu et al. 2019).

Epidemiology Studies—Male Reproductive Development. Studies of DEHP-induced effects on the development of the male reproductive system in humans have examined relationships with cryptorchidism, hypospadias, hydrocele, AGD, and penile size in infants and children and onset of puberty in adolescents (Table 2-15).

Swan (2008) reported an association between decreased probability of normal testicular descent at 1 year of age and MEHP levels in maternal urine (sampled at ~29 weeks of gestation) in a prospective study of

Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Reproductive Development in Males

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Reproductive tract development				
Adibi et al. 2015; Barrett et al. 2016; Martino-Andrade et al. 2016; Swan et al. 2015 Cohort, 366 male newborns, AGD measured shortly after birth, United States (Minnesota, California, New York, Washington)	**Anopenile or anoscrotal distance**	**ΣDEHP**	GM (95% CI): 71.7 (65.6, 78.3) nmol/L	↓
		MEHP	GM (95% CI): 1.93 (1.76, 2.11) ng/mL	↓
		MEHHP	6.04 (5.49, 6.64)	↓
		MEOHP	4.22 (3.84, 4.63)	↓
		MECPP	8.12 (7.42, 8.89)	↔
		Martino-Andrade et al. (2016) reported negative associations between AGD in male infants and maternal urinary metabolites in the first trimester, but not second or third trimester, and between penile width and maternal urinary metabolites in the second trimester, but not first or third trimester.		
Arbuckle et al. 2019 Cohort, 147 male newborns, AGD measured at mean age 3.5 days, Canada	Anopenile or anoscrotal distance	ΣDEHP	Low stress: GM (95% CI): 56.3 (46.5, 68.1) nmol/L	↔
			High stress: 50.3 (41.6, 60.7)	↔
		MEHP	Low stress: 2.0 (1.6, 2.4) ng/mL	↔
			High stress: 1.9 (1.6, 2.3)	↔
		MEHHP	Low stress: 8.4 (6.9, 10.2)	↔
			High stress: 7.0 (5.7, 8.6)	↔
		MEOHP	Low stress: 5.7 (4.8, 6.9)	↔
			High stress: 5.1 (4.2, 6.2)	↔
	The low stress group consisted of women reporting no stressful life events (SLE) or reporting 1 SLE but classified it as not at all stressful during pregnancy. The high stress group consisted of women reporting ≥1 SLE as somewhat, moderately, or very much stressful.			

Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Reproductive Development in Males

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Bornehag et al. 2015 Cohort, 196 male infants, AGD measured at mean age 20.8 months, Sweden	Anopenile or anoscrotal distance	ΣDEHP	IQR: 84.56–220.71 nmol/L	↔
		MEHP	IQR: 1.91–5.86 ng/mL	↔
		MEHHP	8.69–22.85	↔
		MEOHP	5.67–15.60	↔
		MECPP	8.00–22.50	↔
Bustamante-Montes et al. 2013 Cohort, 73 male infants, reproductive measurements 24–48 hours after birth, Mexico	Anoscrotal distance or distance from anus to anterior or posterior base of penis; penile width	MEHP	IQR: 0.4–19.5 ng/mL	↔
	Penile length	MEHP	See above	↓
Chevrier et al. 2012 Nested case-control, 21 cases of hypospadias, 50 cases of cryptorchidism, and (for each) 3:1 matched controls, France	Hypospadias or cryptorchidism	ΣDEHP	NR	↔
		MEHP	5^{th}–95^{th} percentile: 0.8–40.7 ng/mL	NR
		MEHHP	4.6–147.0	NR
		MEOHP	3.6–112.0	NR
		MECPP	11.6–183.0	NR
Jensen et al. 2016 Cohort, 273 male infants, reproductive measurements at age 3 months, Denmark	Anopenile or anoscrotal distance, penile width	ΣDEHP	Molar sum: 11.4–36.1 ng/mL	↔

Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Reproductive Development in Males

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Sathyanarayana et al. 2016a Cohort, 371 males, genital anatomical anomalies evaluated during physical exam at birth, United States (Minnesota, California, New York, Washington)	**Genital anomaly or hydrocele**	**ΣDEHP**	IQR: 14.86–38.80 nmol/L (SG-adj)	↑
		MEHP	IQR: 1.28–3.63 ng/mL (SG-adj)	↑
		MEHHP	3.76–11.24	↑
		MEOHP	2.54–7.25	↑
		MECPP	6.42–16.21	↔
	Hypospadias or cryptorchidism	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	See above	↔
Swan 2008 Cohort, 106 male infants, reproductive measurements at age 12.8 months, United States (Minnesota, Missouri, California) **Swan et al. 2005** reported previous analysis of this cohort (smaller n)	**Distance from anus to cephalad base of penis**	**MEHP**	Short AGD: Median 6.2 ng/mL Intermediate AGD: 2.9 Long AGD: 2.3	↓
		MEHHP	Short AGD: 19.8 Intermediate AGD: 10.0 Long AGD: 8.2	↓
		MEOHP	Short AGD: 21.3 Intermediate AGD: 11.7 Long AGD: 7.3	↓
	Probability of normal testicular descent	ΣDEHP, MEHHP, or MEOHP	NR	↔
		MEHP	See above	↓
	Penile width	ΣDEHP, MEHHP, or MEOHP	NR	↔
		MEHP	See above	↓
	Penile length	ΣDEHP, MEHP, MEHHP, or MEOHP	See above	↔

Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Reproductive Development in Males

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Suzuki et al. 2012 Cohort, 111 male infants, AGD measured at birth, Japan	**Distance from anus to anterior genitalia**	**ΣDEHP**	IQR: 23.20–74.70 ng/mL	↓
Wenzel et al. 2018 Cohort, 171 male newborns, AGD measured within 48 hours of birth, United States (South Carolina)	**Anopenile distance**	ΣDEHP	IQR: 36.3–92.8 nmol/L (SG-adj)	↔
		MEHP	IQR: 1.7–5.3 ng/mL (SG-adj)	↓
		MEHHP	4.5–12.2	↔
		MEOHP	3.8–9.0	↔
	Anoscrotal distance	ΣDEHP, MEHP, MEHHP, or MEOHP	See above	↔
Timing of puberty				
Berger et al. 2018 Cohort, 159 adolescent boys (including 55 normal weight boys and 89 overweight/obese boys), reproductive development assessed every 9 months from age 9–13 years, United States (California)	**Age at genital or pubic hair development (overweight/obese boys)**	**ΣDEHP**	NR	↓
		MEHP	IQR: 2.6–7.6 ng/mL (SG-adj)	NR
		MEHHP	10.9–32.1	NR
		MEOHP	7.7–21.4	NR
		MECPP	20.7–47.0	NR
	Age at genital or pubic hair development (all boys or normal weight boys)	ΣDEHP	See above	↔
Cathey et al. 2020a, 2020b Cohort, 91 adolescent boys, reproductive development evaluated at age 8–14 years (visit 1) and age 9–18 years (visit 2), Mexico	Genital or pubic hair development or Testicular volume	ΣDEHP	NR	↔
		MEHP	GM: 6.18 (SG-adj)	↔
		MEHHP	21.2	↔
		MEOHP	12.2	↔
		MECPP	37.0	↔
	Units were not reported for the GM urinary concentrations.			

Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Reproductive Development in Males

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Ferguson et al. 2014a, 2014d Cohort, 110 adolescent boys, reproductive development evaluated at age 8–14 years, Mexico	**Pubic hair development**	MEHP	IQR: 2.97–9.91 ng/mL	↔
		MEHHP	12.1–37.5	↓
		MEOHP	7.55–21.5	↔
		MECPP	23.4–54.4	↔
	Genital development or Testicular volume	MEHP, MEHHP, MEOHP, or MECPP	See above	↔
Su et al. 2015 Cohort, 59 adolescent boys, testicular volume evaluated at age 8 and 11 years, Taiwan	Testicular volume	ΣDEHP	Mean (SD): 0.94 (2.12) µg/g Cr	↔
		MEHP	0.30 (0.68)	↔
		MEHHP	0.38 (1.03)	↔
		MEOHP	0.25 (0.53)	↔

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; AGD = anogenital distance; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; IQR = interquartile range; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NR = not reported; SD = standard deviation; SG-adj = specific gravity adjusted

male infants in the United States. In a case-control study nested within two large birth cohorts in France, Chevrier et al. (2012) observed no increase in the risk of either hypospadias or cryptorchidism at birth associated with maternal urinary DEHP metabolites. Sathyanarayana et al. (2016a) also did not find an increased risk of hypospadias and cryptorchidism and first trimester maternal urinary DEHP metabolites in male infants from a large birth cohort from four medical centers. However, increased maternal urinary DEHP levels were associated with an increased risk of hydrocele or all male genital anomalies combined. Based on a systematic review of available epidemiological data, NAS (2017) concluded that data are inadequate to evaluate the potential association between fetal exposure to DEHP and hypospadias in humans.

Twelve epidemiological studies have investigated the association between reduced AGD in male infants and prenatal DEHP exposure in eight different birth cohorts at various ages between birth and 2 years of age. Associations between decreased AGD and DEHP metabolite levels in maternal urine have been reported in four birth cohorts (Barrett et al. 2016; Martino-Andrade et al. 2016; Suzuki et al. 2012; Swan 2008; Swan et al. 2015; Wenzel et al. 2018). In addition, the effect estimates in three of the four remaining cohorts (Bornehag et al. 2015; Bustamante-Montes et al. 2013; Jensen et al. 2016) were suggestive of a negative association between AGD (both anoscrotal and anopenile distances) in male infants and prenatal DEHP exposure. This finding was consistent across cohort studies in the United States, Scandinavia, Mexico, and Japan, and across ages from birth to 20 months. No association between AGD at 3.5 days of age and prenatal DEHP exposure was observed in a Canadian birth cohort (Arbuckle et al. 2019).

A meta-analysis of five epidemiological studies (Bornehag et al. 2015; Bustamante-Montes et al. 2013; Jensen et al. 2016; Swan 2008; Swan et al. 2015) reported an approximate 4% decrease in AGD per log-increase in maternal DEHP urinary metabolite concentration (Summary estimate of -4.07, 95% CI: -6.49, -1.66) (NAS 2017). Based on this meta-analysis and a systematic review of available epidemiological data, NAS (2017) concluded that there is a moderate level of evidence that fetal exposure to DEHP is associated with a reduction in AGD in humans; confidence in the body of evidence was also moderate.

In studies examining the effects of DEHP exposure on infant penile dimensions (Bustamante-Montes et al. 2013; Jensen et al. 2016; Martino-Andrade et al. 2016; Swan 2008), results were not consistent. American cohorts reported negative associations between penile width in newborns and 2nd trimester (but not 1st or 3rd trimester) maternal DEHP urinary metabolites (Martino-Andrade et al. 2016) and 1-year-old boys and maternal urinary MEHP levels (Swan 2008). However, no association between penile width

and maternal DEHP metabolites was observed in Mexican newborns (Bustamante-Montes et al. 2013) or Danish infants at 3 months of age (Jensen et al. 2016). Bustamante-Montes et al. (2013) reported an association between reduced penile length in newborn boys and maternal MEHP levels; however, this association was not observed the cohort evaluated by Swan (2008).

Four studies examined the relationship between timing of puberty in boys and maternal DEHP exposure. One cohort of 8–14-year-old boys observed a decreased odds of pubic hair development with increased maternal MEHHP urinary levels (but not other metabolites); no associations were observed for genital development or testicular volume (Ferguson et al. 2014a). No associations between pubic hair development, genital development, and/or testicular volume in boys ages 8–18 years of age were observed in the other cohorts (Berger et al. 2018; Cathey et al. 2020a; Su et al. 2015). However, Berger et al. (2018) observed a decrease in the mean age at pubic hair development with increased prenatal DEHP exposure specifically in overweight or obese boys.

In a cross-sectional study using NHANES (2011–-2012) data, Meeker and Ferguson (2014) observed decreased serum testosterone associated with increased urinary levels of DEHP metabolites in a group of 134 boys ages 6–12 years. In another cross-sectional study, urinary DEHP metabolite levels in Taiwanese boys <12 years of age were not associated with serum testosterone (total or free), FSH, LH, or estradiol and urinary DEHP metabolite levels, but a negative association was observed between SHBG and urinary MEHP levels (but not other metabolites) (Wen et al. 2017). Cross-sectional studies were not included in Table 2-15; no other data on serum reproductive hormone levels in prepubertal boys were located.

Animal Studies—Male Reproductive Development. Only one study evaluated male reproductive development following inhalation exposure. Kurahashi et al. (2005) reported a 2–4-fold increase in plasma testosterone in weanling male Wistar rats intermittently exposed to DEHP at concentrations of 0.3–1.6 ppm for 4 or 8 weeks immediately following weaning. No exposure-related changes were observed in serum LH or follicle stimulating hormone (FSH). Though increased relative seminal vesicle weights were observed after exposure for 8 weeks, no histopathological lesions in the testes were observed. Neither timing of sexual maturation nor sexual performance were evaluated.

In nonhuman primates, no changes in testes/epididymides weights or testicular histology occurred in sexually immature 2-year-old Cynomolgus monkeys that were treated with 500 mg DEHP/kg/day by gavage for 14 consecutive days (Pugh et al. 2000). Similarly, exposure to doses up to 2,500 mg/kg/day

for 65 weeks from weaning at 3 months to sexual maturity at 18 months did not result in changes in serum testosterone, male reproductive organ weight or histology, or sperm parameters in marmoset monkeys (Tomonari et al. 2006).

Permanent reproductive tract malformations and lesions have been observed in rat offspring following gestational plus lactational exposure to DEHP at doses of 3 mg/kg/day or higher. In Wistar rats, an increased incidence of male offspring with mild external genital dysgenesis was observed following maternal exposure to DEHP at doses ≥3 mg/kg/day from GD 7 to PND 16 (lowest dose tested) (Christiansen et al. 2010). In addition, nipple retention was observed at ≥10 mg/kg/day and decreased seminiferous tubule diameter with fewer germ cells and focal Leydig cell hyperplasia occurred at ≥300 mg/kg/day (Christiansen et al. 2010). Another gestation plus lactation exposure study in Wistar rats did not observe hypospadias until 700 mg/kg/day (Venturelli et al. 2019).

In Long-Evans rats, testicular lesions were also observed at maternal doses ≥3 mg/kg/day in offspring exposed to DEHP during gestation and lactation (Arcadi et al. 1998). In Sprague-Dawley rat offspring exposed via maternal doses of 300 mg/kg/day from GD 8 to PND 21, abnormal findings included increased multinucleated gonocytes at PND 3 and increased incidences of hemorrhagic testes at PND 8 (Nardelli et al. 2017). These findings were not observed at ≤30 mg/kg/day, and nipple retention (another demasculinization endpoint) was not observed at doses up to 300 mg/kg/day. In another study in Sprague-Dawley rats, when all reproductive malformations were pooled for analysis, a significant increase in malformed male offspring was observed at maternal exposure levels of ≥11 mg/kg/day during gestation and lactation (a subset of the offspring also received direct DEHP exposure on PNDs 18–64) (Gray et al. 2009). When malformations were evaluated separately, significant findings included abnormal testes histology at 33 and 300 mg/kg/day, malformed coagulating gland at ≥100 mg/kg/day, and permanent nipples and gross testicular and epididymal abnormalities at 300 mg/kg/day.

In a systematic review of available rodent data evaluating hypospadias following oral *in utero* exposure, NAS (2017) concluded that there is a moderate level of evidence that fetal exposure to DEHP is associated with hypospadias in rats; confidence in the body of evidence was also moderate.

Histopathological alterations were also observed in PND 1 and 22 male offspring of Sprague-Dawley rats exposed to doses ≥135 mg/kg/day from GD 6 to PND 21, but not ≤45 mg/kg/day; the changes included enlarged, bi- and multinucleated gonocytes; gonocyte degeneration; acute interstitial hemorrhage and loosening of connective tissue; reduced germ cell differentiation; and hyperemia (Andrade et al. 2006c).

By adulthood, abnormal testicular histological findings were largely limited to grossly abnormal testes in male offspring at 405 mg/kg/day (3/20 “small” scrotal testes, 1/20 undescended testes), along with slight focal Leydig cell hyperplasia in 1/20 males and massive reduction of germ cell layers in 2/20 males at 405 mg/kg/day (Andrade et al. 2006a). However, the majority of seminiferous tubules were unaffected by treatment, and no major malformations were observed at maternal doses up to 405 mg/kg/day (although increased nipple retention was observed at this dose) (Andrade et al. 2006a, 2006c).

In gestational exposure-only studies, increased nipple retention on PND 13 and increased hypospadias and cryptorchidism on PND 63 were observed in Sprague-Dawley rats at 500 mg/kg/day, but not ≤100 mg/kg/day (Vo et al. 2009a). Increased nipple retention was also observed in F1 and F2 pups at ≥1,040 mg/kg/day, but not ≤380 mg/kg/day, in 2-generation studies in Wistar rats (Schilling et al. 1999, 2001). No change in testes histology was observed in male rat offspring at PND 3, 90, or 120 following maternal exposure to doses up to 10 mg/kg/day from GD 14 through parturition (Walker et al. 2020). Abdel-Maksoud et al. (2019) qualitatively reported histopathological changes in Long-Evans rat offspring at PND 35 after maternal exposure to 0.05 mg/kg/day on GDs 12–21, including focal germ cell loss, sloughing of germ cells, and diffuse interstitial cell hyperplasia. However, due to lack of quantitative data, these findings cannot be independently reviewed for NOAEL/LOAEL determination. Therefore, it is not included in the LSE table.

In mice, an increased incidence of hypospadias was observed in C57BL/6 mouse fetuses at GD 19 following maternal exposure to doses ≥100 mg/kg/day (lowest dose tested) from GD 12 to 17 (Liu et al. 2008). Decreased anterior urethra length in male fetuses was observed at ≥200 mg/kg/day. Similarly, in CD-1 mice exposed from GD 11 until PND 0, no external malformations at birth or histopathological changes in the testes of epididymides at PND 21 or 60 were observed at maternal doses up to 750 mg/kg/day (Barakat et al. 2017). When male offspring were evaluated at 22 months of age, histopathological changes, including hypospermatogenesis, germ cell degeneration, fewer developing spermatids, abnormal residual bodies in the lumen, and presence of epididymal vacuoles and germ cells in lumen of epididymis, were observed in surviving mice at ≥0.2 mg/kg/day. These effects were not evaluated at the low dose (0.02 mg/kg/day), because all low-dose mice died prior to 22 months of age (the cause of death was undetermined, but was unlikely to be related to treatment due to survival in higher dose groups). Due to premature death of all low-dose animals, a reliable NOAEL/LOAEL could not be established for this study. Therefore, Barakat et al. (2017) is not included in the LSE table.

Changes in fetal testicular histopathology were also observed following gestational exposure to DEHP. In Sprague-Dawley and Long-Evans rats, gestational exposure to maternal doses ≥10 mg/kg/day (lowest dose tested) resulted in Leydig cell clustering in fetal testes (Klinefelter et al. 2012; Lin et al. 2008, 2009). At maternal doses ≥100 mg/kg/day, dysgenic seminiferous cords were also observed. In Wistar rats, Leydig cell clustering was also observed in GD 21 offspring after maternal exposure to ≥100 mg/kg/day from GD 7 to 21, but not ≤30 mg/kg/day (Borch et al. 2006). Additional effects observed at maternal doses ≥100 mg/kg/day included multinucleated gonocytes, increased gonocyte number, and centralized gonocytes, and Sertoli cell vacuolization (Borch et al. 2006). However, in GD 18.5 C57Bl/6 × B6129S4 mouse offspring, no changes in the number of germ cells were observed following maternal exposure to doses up to 250 mg/kg/day from GD 7 to 16 (Ungewitter et al. 2017).

Alterations in male reproductive organ histology have also been reported in neonatal rats exposed directly to DEHP. Loss of spermatocytes and decreased number of Sertoli cells have been observed in Sprague-Dawley rats exposed to DEHP for 5 days during early postnatal development (PNDs 6–10 or 14–18) or post-weaning (PNDs 21–25 or 42–46) at doses ≥1,000 mg/kg/day, but not ≤100 mg/kg/day; rats were sacrificed 24 hours after the final dose (Dostal et al. 1988). Altered morphology of germ cells (mitotic alterations in gonocytes and/or enlarged and multinucleated gonocytes) were observed in male Sprague-Dawley rats following exposure to ≥100 mg/kg/day on PND 3 or ≥60 mg/kg/day from PND 3 to 7; reduced Sertoli cell proliferation and apoptosis observed at ≥100 mg/kg/day (Camacho et al. 2020; Li et al. 2000). With exposure on PNDs 3–23, decreased seminiferous tubule diameter was observed at ≥60 mg/kg/day with decreased testicular area and increased severity of germinal cell depletion and Sertoli cell vacuolization at ≥300 mg/kg/day (Camacho et al. 2020).

In weanling Sprague-Dawley rats, exposure to ≥10 mg/kg/day from PND 21 to 35 resulted in degeneration of the Leydig cells and “disorders of germ cells” in the testes of young Sprague-Dawley rats (Vo et al. 2009b). Dilatation of the tubular lumen and stratification of germ cells was also observed at ≥100 mg/kg/day. Similarly, decreased thickness and vacuolization of the seminiferous epithelium were observed in weanling Sprague-Dawley rats exposed to ≥150 mg/kg/day from PND 22 to 35; this progressed to severe vacuolization and lack of spermatids in tubules at 450 mg/kg/day (Zhang et al. 2018a). Noriega et al. (2009) also reported hypospermia and testicular and epididymal degeneration in weanling Sprague-Dawley rats at exposure levels ≥300 mg/kg/day, but not ≤100 mg/kg/day. These effects were only observed in similarly exposed Long-Evans rats at 900 mg/kg/day (Noriega et al. 2009). In Wistar weanling rats, however, testicular germ cell damage was observed after exposure to 250 mg/kg/day on PNDs 25–54, but not doses ≤100 mg/kg/day (Parmar et al. 1995). In other studies of

weanling rats, no changes in testicular or seminal vesicle histology were observed in Long-Evans rats exposed to doses up to 200 mg/kg/day for 14–28 days (Akingbemi et al. 2001).

One study reported a variety of histopathological changes in the testes and epididymides in Wistar rats exposed to very low doses of ≥0.0005 mg/kg/day starting at weaning (PND 21) through PND 120 (Oudir et al. 2018). Observed histopathological effects at PND 120 included decreased spermatozoa in testes and increased oligospermia and scattered cellular dispersion in epididymides at ≥0.0005 mg/kg/day. These effects did not show clear dose-response relationships at the lower doses; for example, spermatozoa were found in 80, <50, 60, and <25% of seminiferous tubules in control through high dose animals, and severe oligospermia was found in 2/10 animals in each of the 0.0005 and 5 mg/kg/day groups but not in any animals at 0.05 mg/kg/day. Similarly, sperm counts were significantly decreased by approximately 41 and 46% (compared to control) at 0.0005 and 5 mg/kg/day, respectively, but not at 0.05 mg/kg/day. Leydig cell number was significantly increased at 0.05 mg/kg/day, but not changed in other groups. Sertoli cell counts were decreased by 22 and 42% at 0.05 and 5 mg/kg/day, respectively. None of the developmental studies discussed above evaluated doses as low as the ones evaluated by Oudir et al. (2018), and several reported similar effects only at higher doses. For example, Parmar et al. (1995) did not observe any testicular germ cell changes in Wistar rats exposed on PNDs 25–54 to doses as high as of 100 mg/kg/day. Additionally, Hsu et al. (2016) did not observe sperm effects in Sprague-Dawley rats at a dose of 0.03 mg/kg/day following exposure from PND 42 to 105 (see Section 2.16 for more details). Based on the poor evidence for a dose-response relationship at low doses and lack of corroborating findings of male reproductive effects following exposure to very low DEHP exposures, this study (Oudir et al. 2018) is not included in the LSE table.

Numerous studies have also reported decreased testicular weights following gestational and/or lactational exposure to DEHP, although results were not consistent between species, strains, and studies. In Long-Evans rats, significant decreases in testes weight were observed in offspring following maternal exposure to doses ≥100 mg/kg/day during gestation (Lin et al. 2008) or ≥3 mg/kg/day during gestation and lactation (Arcadi et al. 1998). Some gestational and/or lactational studies in Sprague-Dawley rats reported no changes in offspring testes weights at doses up to 405 mg/kg/day (Andrade et al. 2006a, 2006c; Kobayashi et al. 2006; Nardelli et al. 2017; Walker et al. 2020), while Gray et al. (2009) reported significant decreases at 300 mg/kg/day, but not 33 mg/kg/day. Following postnatal exposure in Sprague-Dawley rats for 5 days starting on PND 6, 14, 21, or 42, doses ≥1,000 mg/kg/day resulted in decreased testes weights, but doses ≤100 mg/kg/day did not (Dostal et al. 1988). With 5- or 21-day postnatal

exposure beginning on PND 3, decreased testes weights were observed in Sprague-Dawley rats at ≥60 mg/kg/day (Camacho et al. 2020).

When Christiansen et al. (2010) conducted two separate experiments in Wistar rats, decreased testes weight was observed in one study at maternal doses ≥10 mg/kg/day, but not at doses up to 100 mg/kg/day in the second study. Decreased testes weight was observed in Wistar rat offspring at 30 mg/kg/day in two additional gestation plus lactation exposure studies (Carbone et al. 2010, 2012), but not at doses up to 500–700 mg/kg/day in others (Dalsenter et al. 200; Venturelli et al. 2019). No changes in testicular weights were observed in F1 or F2 weanlings in a 2-generation study in Wistar rats at doses up to 1,088 mg/kg/day (Schilling et al. 2001) or in PND 92 Wistar rats following lactation exposure to doses up to 75 mg/kg/day (Venturelli et al. 2015).

Three mouse developmental studies evaluated testicular weight in offspring. Pocar et al. (2012) observed that testicular weights were significantly decreased by 13% in CD-1 mouse offspring following maternal exposure to 0.05 mg/kg/day during gestation and lactation but were comparable to controls at 5 mg/kg/day (highest dose evaluated). Following gestation-only exposure, testicular weights were decreased in CD-1 mouse offspring in one study at maternal doses ≥50 mg/kg/day (Do et al. 2012).

Decreased organ weights have also been observed in other male reproductive organs following gestational and/or lactational exposure in some studies. There was a decrease in ventral prostate weight observed in the offspring of rats and mice exposed to DEHP during gestation and lactation at ≥10 mg/kg/day (Christiansen et al. 2010). Dalsenter et al. (2006) and Gray et al. (2009) also observed decreased ventral prostate as well as seminal vesicle weights in rats at 500 and 300 mg/kg/day, respectively. An additional study reported decreased seminal vesicle weights at low exposure levels ≥0.05 mg/kg/day (Pocar et al. 2012). Decreased LABC muscles weights were observed in two studies following maternal exposure to ≥10 mg/kg/day (Christiansen et al. 2010) and 300 mg/kg/day (Gray et al. 2009). Gray et al. (2009) also reported decreased weights of the glans penis, Cowper's glands, and epididymides at 300 mg/kg/day. Epididymal weights were also reportedly decreased at ≥0.1 mg/kg/day in a study by Wang et al. (2017a).

In other studies, no changes in other male reproductive organs were observed in Sprague-Dawley rats exposed during gestation and lactation to maternal doses up to 405 mg/kg/day (Andrade et al. 2006a, 2006c; Kobayashi et al. 2006, Nardelli et al. 2017). Similarly, there were no male reproductive organ changes in F1 or F2 weanlings in a 2-generation study in Wistar rats at doses up to 1,088 mg/kg/day (Schilling et al. 2001). In other studies in Wistar rats, no changes in male reproductive organs were

observed at PNDs 90–92 following lactation exposure to doses up to 75 mg/kg/day or gestation plus lactation exposure up to 700 mg/kg/day (Venturelli et al. 2015, 2019).

Altered male reproductive organ weights have also been reported in young rats following exposure to DEHP after weaning. The lowest level observed for decreased testes weight was 10 mg/kg/day when Sprague-Dawley rats were exposed for 15 days post-weaning (Vo et al. 2009b). Other studies indicated decreased reproductive organ weight in young Sprague-Dawley, Long-Evans, or Wistar rats exposed to ≥100 mg/kg/day for 14–76 days post-weaning (Noriega et al. 2009; Parmar et al. 1995; Zhang et al. 2018a). In other Long-Evans rat studies, decreased testicular weights were observed from exposure to 500 mg/kg/day from PND 21 to 34, but not after exposure to doses ≤200 mg/kg/day for 28–100 days starting at PND 21 or 35 (Akingbemi et al. 2001, 2004; Ge et al. 2007). Yet another study in young Long-Evans rats showed a non-monotonic response to DEHP exposure from PND 21 to 48, with increased weight of the seminal vesicles at 10 mg/kg/day, but decreased weight of the seminal vesicles, prostate, and testes at 750 mg/kg/day (Ge et al. 2007). In young Wistar rats, changes in the weights of the seminal vesicles, ventral prostate, epididymis, or testes were not observed following exposure to doses up to 75 mg/kg/day for 30 days post-weaning (Venturelli et al. 2015).

Decreased AGD, suggesting demasculinization, has been reported in male rat offspring following gestational and/or lactational exposure to DEHP. AGD was significantly decreased in PND 0 male offspring of Long-Evans rat dams exposed to DEHP from GD 2 to 20 at 750 mg/kg/day (Lin et al. 2008). Similarly, AGD was significantly decreased in PND 21 male offspring of Long-Evans rat dams exposed to DEHP from GD 12.5 to PND 21.5 at 750 mg/kg/day (Lin et al. 2009). In Sprague-Dawley rats, AGD and or and the anogenital index (AGI; corrected for body weight) were significantly decreased at PNDs 2–3 following gestational and lactational exposure to ≥300 mg/kg/day, but not at doses up to 135 mg/kg/day (Andrade et al. 2006c; Gray et al. 2009; Nardelli et al. 2017). Decreased AGD was also observed in PND 63 male offspring of Sprague-Dawley rat dams exposed to DEHP from GD 11 to 21 at ≥100 mg/kg/day. No changes in AGD were observed in PND 3 or 120 male offspring of Sprague-Dawley rat dams exposed to doses up to 10 mg/kg/day from GD 10 until parturition (Walker et al. 2020). Gestation/lactation exposure studies in Wistar rats reported decreased AGD at PND 1 at doses ≥10 mg/kg/day (Christiansen et al. 2010) and decreased AGI at PND 4 at 700 mg/kg/day (Venturelli et al. 2019).

Multigeneration studies have equivocal results for AGD and AGI. In a 2-generation study in Wistar rats, both AGD and AGI were significantly decreased on PND 1 or 2 in both F1 and F2 males at doses

≥340 mg/kg/day (Schilling et al. 2001). These findings were not observed until doses of 1,040 mg/kg/day in an earlier study by the same group (Schilling et al. 1999). In a 3-generation study in Wistar rats, AGD, but not AGI, was decreased in F1, F2, and F3 male pups on PND 1 at 447 mg/kg/day, but not ≤57 mg/kg/day (Blystone et al. 2010; NTP 2005).

Studies in mice generally do not show changes in AGD or AGI. One study reported decreased AGD in C57BL/6 mouse fetuses at GD 19 following maternal exposure to doses ≥100 mg/kg/day (lowest dose tested) from GD 12 to 17 (Liu et al. 2008). However, no exposure-related changes in AGD were observed in CD-1 mouse offspring following gestational exposure up to 500 mg/kg/day (Do et al. 2012), or gestation plus lactation exposure to doses up to 5 mg/kg/day (Pocar et al. 2012). In C57BL/6J × FVB offspring, no exposure-related changes in AGD were observed following maternal exposure to doses up to 100 mg/kg/day from 2 weeks premating through lactation (Bastos Sales et al. 2018). In C57BL/6J × B6129S4 offspring, there was a significant increase, as opposed to a decrease, in AGI at GD 18.5 following gestational exposure to 250 mg/kg/day (Ungewitter et al. 2017).

A meta-analysis of 13 gestational oral studies in rats reported a statistically significant overall effect reduction in AGD with DEHP exposure (-3.96; 95% CI -5.07, -2.85) (NAS 2017). A meta-analysis of three gestational oral studies in mice was also conducted, but an overall significant effect was not observed. However, linear regression analyses showed statistically significant decreases in AGD of ~2% per unit DEHP dose or log-transformed dose in both rats and mice. BMD_5 values of 270 and 110 mg/kg/day were identified for rats and mice, respectively. Based on these meta-analyses and a systematic review of available rodent data evaluating AGD following oral in utero exposure, NAS (2017) concluded that there is evidence that fetal exposure to DEHP is associated with a reduction in AGD in rats; confidence in the body of evidence was high.

In multigenerational studies in rats, delayed preputial separation (PPS) was observed in male offspring exposed to doses ≥447 mg/kg/day, but not ≤380 mg/kg/day (Blystone et al. 2010; NTP 2005; Schilling et al. 1999, 2001). Delayed puberty may be due to developmental exposure, peripubertal exposure, or a combination of the two; it may also be secondary to decreased body weights observed at the same doses. However, PPS was also significantly delayed in male offspring of Sprague-Dawley rats exposed to doses ≥15 mg/kg/day from GD 6 to PND 21 in the absence of decreased body weights (Andrade et al. 2006c). PPS was also significantly delayed in male offspring of Wistar rats exposed to 700 mg/kg/day from GD 13 to PND 21, despite transient increases in body weight during lactation and early postweaning periods (Venturelli et al. 2019). Delayed PPS was also reported in Sprague-Dawley and Long-Evans rats

exposed to ≥300 mg/kg/day for 22–76 days immediately following weaning, but not ≤100 mg/kg/day (Noriega et al. 2009). Another study in young Long-Evans rats showed a non-monotonic response to DEHP exposure from PND 21 to 48, with decreased age of PPS at 10 mg/kg/day, but increased age of PPS at 750 mg/kg/day (Ge et al. 2007). The significance of this non-monotonic response is unclear.

Other studies did not observe any exposure-related changes in the age at PPS in male rat offspring following maternal exposure to doses up to 500 mg/kg/day during gestation and/or lactation (Dalsenter et al. 2006; Gray et al. 2009; Nardelli et al. 2017; Venturelli et al. 2015), direct exposure to doses up to 150 mg/kg/day on PNDs 6–96 (Kim et al. 2018c), or direct exposure to doses up to 75 mg/kg/day on PNDs 22–52 (Venturelli et al. 2015). A subset of the offspring also received direct DEHP exposure on PNDs 18–64; PPS was not delayed in these rats either (Gray et al. 2009).

In a 2-generation study in Wistar rats, loss of spermatocytes was observed in 2/10 weanling F1 rats at 360 mg/kg/day and 7/9 weanling F1 rats at 1,040 mg/kg/day; no changes in spermatocytes were observed at 130 mg/kg/day (Schilling et al. 1999). Changes in sperm parameters have also been observed in adult rat offspring following gestational exposure to doses ≥10 mg/kg/day (Vo et al. 2009a) and gestational plus lactational exposure to doses ≥3 mg/kg/day (Andrade et al. 2006a; Arcadi et al. 1998). Sperm effects included decreased sperm concentration, viability, and motility; decreased daily sperm production; and altered morphology (elongated or round spermatids). Whole sperm count was also decreased in adult rat offspring following gestational, lactational, and post-lactational exposure to DEHP at 300 mg/kg/day through PND 65, but not at doses ≤100 mg/kg/day (Gray et al. 2009).

Sperm count and viability were decreased approximately 50 and 20%, respectively, in PND 42 offspring of CD-1 mouse dams exposed to 0.05 or 5 mg/kg/day during gestation and lactation (Pocar et al. 2012). Sperm from exposed offspring were capable of fertilizing unexposed oocytes *in vitro* (no change in cleavage rate); however, blastocyst rate was significantly reduced at maternal doses ≥0.05 mg/kg/day (Pocar et al. 2012). Consistent with these *in vitro* fertilization data, no changes in male mating behavior or fertility were observed in adult offspring of Sprague-Dawley rats exposed to DEHP at doses up to 405 mg/kg/day from GD 6 to PND 21 (Andrade et al. 2006a). No changes in male fertility were observed in offspring of female Sprague-Dawley rats exposed to doses up to 10 mg/kg/day from GD 14 though parturition (Walker et al. 2020). Similarly, no change in reproductive performance was observed in CD-1 mouse offspring exposed to doses up to 95 mg/kg/day from GD 0 to 17 (NTP 1988).

At higher doses, sexual behavior was significantly altered in adult male offspring of Wistar rats exposed to 500 mg/kg/day during gestation and lactation, but not at doses ≤100 mg/kg/day (Dalsenter et al. 2006). Observed effects included decreased ejaculation, increased intromission latency, and increased numbers of intromissions until ejaculation. These alterations were accompanied by decreased sperm number and daily sperm production at puberty and adulthood (Dalsenter et al. 2006). No changes in sperm morphology were observed.

Another study with gestation-only exposure reported an increase in the percentage of abnormal sperm in 22-month-old CD-1 mouse offspring at ≥0.2 mg/kg/day, including sperm head, neck, and midpiece, and tail abnormalities (Barakat et al. 2017). At the highest dose (750 mg/kg/day), sperm concentration and motility were reduced by approximately 45 and 35%, respectively. Fertility was also significantly reduced by 61% at 750 mg/kg/day when evaluated at 19 months of age, but not 4, 5, or 7 months of age, suggesting early senescence at this dose. However, since all low-dose (0.02 mg/kg/day) mice died prior to study termination, a reliable NOAEL/LOAEL cannot be established. Therefore, this study is not included in the LSE table.

In a study with a non-traditional design, male reproductive effects in third-generation (F3) CD-1 offspring were evaluated following exposure 0.02 or 0.2 mg/kg/day in F0 dams only from GD 11 until parturition (no direct exposure to F1, F2, or F3 mice) (Barakat et al. 2020). Fertility percentage was significantly decreased by 33% in paternal lineage F3 males in the 0.02 mg/kg/day group; however, fertility was comparable to control at 0.2 mg/kg/day in the paternal lineage and at both doses in the maternal lineage. Decreased fertility only at the low dose in the paternal lineage is difficult to interpret, as is decreased severity of other observed reproductive effects in paternal lineage males at the high dose, compared to the low dose, including decreased serum testosterone, sperm effects, and testicular lesions. Maternal lineage F3 offspring showed normal fertility, but decreased serum testosterone and sperm concentration were observed at 0.02 mg/kg/day and germ cell degeneration was observed at 0.2 mg/kg/day. Based on low animal number (n=4/dose), lack of clear dose-response, and evaluation only in F3 animals (following exposure in F0 generation only), this study is not included in the LSE table.

Decreased serum testosterone and LH were observed in GD 21 Sprague-Dawley rat offspring following maternal exposure to 500 mg/kg/day during gestation (Vo et al. 2009a) and in PND 15 Wistar rat offspring following maternal exposure to 30 mg/kg/day during gestation and lactation (Carbone et al. 2012). Serum testosterone was significantly decreased by >50% in PND 60 male offspring of Sprague-Dawley rat dams exposed to DEHP from GD 14 to PND 0 at doses ≥100 mg/kg/day (Culty et al. 2008;

Martinez-Arguelles et al. 2011). Using the same exposure parameters, Walker et al. (2020) did not observe changes in serum testosterone at PND 3, 90, or 120 at doses up to 10 mg/kg/day. Following gestational and lactational exposure, serum testosterone was significantly decreased by >30% in male PND 21 Long-Evans rats or adult Wistar rats at maternal doses ≥10 mg/kg/day, respectively (Dalsenter et al. 2006; Lin et al. 2009; Rajagopal et al. 2019a).

No exposure-related changes were observed in serum estradiol in PND 60 male offspring at maternal doses up to 1,250 mg/kg/day (Culty et al. 2008; Martinez-Arguelles et al. 2011) or at PND 3, 90, or 120 male offspring at maternal doses up to 10 mg/kg/day (Walker et al. 2020). However, a significant >30% decrease in serum estradiol in adult male Wistar rat offspring rats at maternal doses ≥10 mg/kg/day was reported by Rajagopal et al. (2019a).

In other studies, no exposure-related changes in serum testosterone, estradiol, FSH, or LH were observed in weanling and/or adult male offspring following maternal exposure to doses up to 300 mg/kg/day during gestation and lactation in Sprague-Dawley rats (Gray et al. 2009; Nardelli et al. 2017). A subset of the offspring also received direct DEHP exposure from PND 18 to 64; serum hormone changes were not observed in these rats either (Gray et al. 2009). In Wistar rats, serum FSH was significantly decreased by 33% in PND 30 male offspring following maternal exposure to 30 mg/kg/day during gestation and lactation; this was not observed at 3 mg/kg/day (Carbone et al. 2010). No exposure-related changes in serum LH were observed at maternal doses up to 30 mg/kg/day (Carbone et al. 2010).

In a gestation-only exposure study in CD-1 mice, serum testosterone levels at PND 21 were similar between the groups; however, at 16 months, testosterone levels significantly decreased by approximately 97, 85, 66, and 63% in mice at 0.02, 0.2, 500, and 750 mg/kg/day DEHP, respectively, compared to control (Barakat et al. 2017). The significance of this non-dose-related response is unclear without further study of the pituitary-testes axis; therefore, this study is not included in the LSE table. When evaluated in similarly exposed CD-1 mouse offspring at 22 months, testosterone levels were significantly decreased by 82 and 72% at 500 and 750 mg/kg/day, respectively; findings were not significant at 0.2 mg/kg/day (Barakat et al. 2018). Other reproductive hormones were also altered in aged offspring, included increased serum estradiol at 16 months at 750 mg/kg/day and increased serum LH concentrations at 22 months at ≥500 mg/kg/day (Barakat et al. 2017). Low-dose mice (0.02 mg/kg/day) were not examined at 22 months due to premature death of all animals.

Alterations in male reproductive hormones following post-weaning exposure to DEHP are inconsistent. One study in weanling Long-Evans rats showed a non-monotonic response to DEHP exposure from PND 21 to 48, with increased serum testosterone at 10 mg/kg/day, but decreased serum testosterone at 750 mg/kg/day (Ge et al. 2007). Similarly, serum LH was increased in Sprague-Dawley rats exposed to 900 mg/kg/day for 22, 42, or 76 days post-weaning, but decreased in weanling Sprague-Dawley rats exposed to 900 mg/kg/day for 35 days (Noriega et al. 2009). No exposure-related changes were observed in similarly exposed Long-Evans rats (Noriega et al. 2009). However, other studies in Long-Evans rats reported that exposure to gavage doses ≥10 mg/kg/day for 28–100 days starting at weaning resulted in increased serum LH and testosterone levels and decreased basal and LH-stimulated Leydig cell testosterone production (Akingbemi et al. 2001, 2004). Reduced testosterone production in Leydig cells was also observed following 14-day exposures to ≥10 or 100 mg/kg/day starting on PND 21 or 35, respectively (Akingbemi et al. 2001). No changes in serum hormone levels were observed at doses up to 200 mg/kg/day using the same exposure paradigms (Akingbemi et al. 2001). In Sprague-Dawley rats, serum testosterone was significantly decreased following exposure to ≥10 mg/kg/day for 15 days immediately after weaning, but no changes in serum LH were observed at doses up to 500 mg/kg/day (Vo et al. 2009b).

Fetal serum testosterone was significantly elevated, compared with control, in CD-1 mouse offspring following maternal exposure to 0.0005, 0.005, and 0.5 mg/kg/day from GD 9 to 18; however, serum testosterone in male fetuses at maternal doses of 50 and 500 mg/kg/day were comparable to control (Do et al. 2012). The biological relevance of the non-monotonic dose response relationship for fetal testosterone is also unclear without further study of the pituitary-testes axis.

Decreased levels of fetal testicular testosterone (FTT) were observed in offspring of Wistar rat dams exposed to 300 mg/kg/day from GD 7 to 21 (Borch et al. 2006). In Long-Evans rats exposed from GD 2 to 20, decreased FTT was observed at maternal doses of 10 mg/kg/day, but increased FTT was observed at maternal doses of 750 mg/kg/day (Lin et al. 2008). Intratesticular testosterone levels were not altered on PND 1 in Sprague-Dawley rats exposed from GD 6 to PND 1 to doses up to 405 mg/kg/day (Andrade et al. 2006c). In Sprague-Dawley weanling rats, testicular testosterone production was decreased following exposure to doses ≥300 mg/kg/day for 22–76 days post-weaning (Noriega et al. 2009). *Ex vivo* FTT production was decreased by >20% following maternal exposure to DEHP for 5–15 days during gestation at doses ≥50 mg/kg/day in Sprague-Dawley rats (lowest dose tested) and ≥300 mg/kg/day in Wistar rats (Borch et al. 2006; Furr et al. 2014; Hannas et al. 2011; Howdeshell et al. 2008; Klinefelter et al. 2012; Saillenfait et al. 2013). FTT production was decreased by >90% at 900 mg/kg/day. No changes

in FTT production were observed in GD 18 fetuses of CD-1 mouse dams exposed to doses up to 500 mg/kg/day from GD 9 to 18 (Do et al. 2012) or GD 18.5 fetuses of C57Bl/6 × B6129S4 mouse offspring following maternal exposure to doses up to 250 mg/kg/day from GD 7 to 16 (Ungewitter et al. 2017).

A meta-analysis of seven gestational oral studies in rats reported a statistically significant overall effect for reduced fetal testicular testosterone and DEHP exposure (-110.14; 95% CI -136.73, -83.54) (NAS 2017). Linear regression analyses also showed statistically significant associations. A BMD_5 value of 15 mg/kg/day was calculated. In addition, an alternate BMD_{40} value of 160 mg/kg/day was calculated. An alternate of benchmark response (BMR) of 40% was selected because this level is assumed to be biologically relevant based on previous studies showing reproductive tract malformations in male rats when fetal testosterone production was reduced by about 40%. Based on this meta-analysis and a systematic review of available rodent data evaluating fetal testosterone levels following oral *in utero* exposure, NAS (2017) concluded that there is a high level of evidence that fetal exposure to DEHP is associated with a reduction in fetal testosterone in rats; confidence in the body of evidence was high.

Altered hormone levels may be due to Leydig cell toxicity. Sex hormone production (testosterone, estradiol) by Leydig cells, measured *ex vivo*, was significantly altered in cells harvested from young rats exposed at doses ≥10 mg/kg/day for 14–100 days after weaning. Across time, the direction of alteration (reduced or increased) for hormone production was not consistent, suggesting different potential reproductive effects dependent on exposure timing (e.g., PND 21 or 62) (Akingbemi et al. 2001, 2004). Inhibition of steroidogenic enzyme activities was also observed in rats exposed for 28 days, including reduced 17β-hydroxysteroid dehydrogenase (17β-HSD) at ≥10 mg/kg/day, reduced P450scc and 3β-HSD at ≥100 mg/kg/day, and reduced P45017α at 200 mg/kg/day (Akingbemi et al. 2001). In another study, young rats exposed from PND 21 to 34 also showed decreased testosterone production by Leydig cells cultured *in vitro*, but only in cells from animals exposed to 500 mg/kg/day, not 10 mg/kg/day (Ge et al. 2007).

Mechanisms of Altered Male Reproductive Development. The anti-androgenic effects of DEHP do not appear to be mediated by the androgen receptor (AR), because neither DEHP nor MEHP bind the human AR *in vitro* (Parks et al. 2000). Alterations in the hypothalamic-pituitary axis may underlie some of the observed effects in the developing male reproductive system. Carbone et al. (2010, 2012) reported decreased aspartate and increased GABA in the hypothalamus of male offspring of Wistar rats exposed to 30 mg/kg/day during gestation and lactation. These changes could account for observed decreases in

serum testosterone, LH, and FSH levels (via decreased release of gonadotropin releasing hormone) in male offspring at this exposure level. Several studies suggest that oxidative stress and inflammatory processes (i.e., macrophage infiltration and cytokine production) play a role in testicular toxicity induced by DEHP or MEHP in neonatal or prepubertal rats (Stermer et al. 2017; Tang et al. 2019; Voss et al. 2018; Zhang et al. 2017, 2020e).

Numerous studies have reported alterations in gene expression related to testicular functions including testicular descent (insulin-like factor 3 or *Insl3*), cholesterol transport (*Scarb1*, *Star*), steroid biosynthesis (*CYP11a1, Hsd3b1, CYP17a1*), and Sertoli-gonocyte interaction (*c-kit*) (Albert and Jégou 2014; Arzuaga et al. 2019; Dorman et al. 2018; NAS 2017). Prenatal exposure to DEHP also altered the expression of genes related to sexual differentiation in the epididymis (AR, ER, Wnt4, β-catenin, MAPK, HOXD4) (Abdel-Maksoud et al. 2018). Time course experiments using fetal and neonatal rat testes cultures exposed to MEHP showed that Leydig cells were affected first, resulting in a decrease in the germ cell pool, followed by decreased Sertoli cell proliferation and function (i.e., decreased secretion of anti-Mullerian hormone) (Albert and Jégou 2014).

MEHP-induced effects in *in vitro* test systems using cultured testes, Sertoli cell cultures, or mixed Sertoli cell and germ cell cultures include altered morphology of testes and seminiferous tubules (Chauvigné et al. 2009), decreased gonocyte numbers and increased numbers of apoptotic gonocytes (Chauvigné et al. 2009), increased germ cell detachment from Sertoli cell surfaces (Gray and Beamand 1984; Gray and Gangolli 1986; Sjöberg et al. 1986), decreased germ cell viability (Gray and Beamand 1984), elongation of Sertoli cells without evidence of decreased viability (Gray and Beamand 1984), decreased FSH binding to Sertoli cells (Grasso et al. 1993), decreased Sertoli cell proliferation (Li and Kim 2003; Li et al. 1998), decreased anti-Müllerian hormone production by Sertoli cells (Chauvigné et al. 2009), decreased testosterone production (Chauvigné et al. 2009; Jones et al. 1993), increased lactate/pyruvate ratio and decreased cellular ATP levels (Heindel and Powell 1992; Moss et al. 1988), decreased expression of selected Sertoli cell proteins (Li and Kim 2003), and destruction of Sertoli cell tight junctional structure (Zhang et al. 2008).

Epidemiology Studies—Female Reproductive Development. AGD in female infants has been assessed in three pregnancy cohorts (Adibi et al. 2015; Arbuckle et al. 2019; Barrett et al. 2016; Swan et al. 2015; Wenzel et al. 2018). No clear associations between maternal urinary DEHP metabolites and female infant anoclitoral or anofourchette distance were observed in any cohort (Table 2-16).

Table 2-16. Summary of Epidemiological Studies of DEHP Exposure and Reproductive Development in Females

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Reproductive tract development				
Adibi et al. 2015; Barrett et al. 2016; Swan et al. 2015 Cohort, 373 female newborns, AGD measured shortly after birth, United States (Minnesota, California, New York, Washington)	Anoclitoral or anofourchette distance	ΣDEHP	Maternal GM (95% CI): 71.7 (65.6, 78.3) nmol/L	↔
		MEHP	GM (95% CI): 1.93 (1.76, 2.11) ng/mL	↔
		MEHHP	6.04 (5.49, 6.64)	↔
		MEOHP	4.22 (3.84, 4.63)	↔
		MECPP	8.12 (7.42, 8.89)	↔
Arbuckle et al. 2019 Cohort, 153 female newborns, AGD measured at mean age 3.5 days, Canada	Anoclitoris or anofourchette distance	ΣDEHP	Maternal: Low stress: GM (95% CI): 56.3 (46.5, 68.1) nmol/L	↔
			High stress: 50.3 (41.6, 60.7)	↔
		MEHP	Low stress: 2.0 (1.6, 2.4) ng/mL	↔
			High stress: 1.9 (1.6, 2.3)	↔
		MEHHP	Low stress: 8.4 (6.9, 10.2)	↔
			High stress: 7.0 (5.7, 8.6)	↔
		MEOHP	Low stress: 5.7 (4.8, 6.9)	↔
			High stress: 5.1 (4.2, 6.2)	↔
	The low stress group consisted of women reporting no stressful life events (SLE) or reporting 1 SLE but classified it as not at all stressful during pregnancy. The high stress group consisted of women reporting ≥1 SLE as somewhat, moderately, or very much stressful.			
Wenzel et al. 2018 Cohort, 128 female newborns, AGD measured within 48 hours of birth, United States (South Carolina)	Anoclitoral or anofourchette distance	ΣDEHP	Maternal IQR: 36.3–92.8 nmol/L (SG-adj)	↔
		MEHP	IQR: 1.7–5.3 ng/mL (SG-adj)	↔
		MEHHP	4.5–12.2	↔
		MEOHP	3.8–9.0	↔

Table 2-16. Summary of Epidemiological Studies of DEHP Exposure and Reproductive Development in Females

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Timing of puberty				
Berger et al. 2018 Cohort, 165 adolescent girls (including 84 normal weight and 81 overweight/obese girls), reproductive development assessed every 9 months from age 9 to 13 years, United States (California)	**Age at breast development or menarche (all, normal weight, or overweight/obese)**	**ΣDEHP**	NR	↑
		MEHP	Maternal IQR: 2.6–7.6 ng/mL (SG-adj)	NR
		MEHHP	10.9–32.1	NR
		MEOHP	7.7–21.4	NR
		MECPP	20.7–47.0	NR
	Age at pubic hair development (all, normal weight, or overweight/obese)	ΣDEHP	See above	↔
Binder et al. 2018a, 2018b Cohort, 200 adolescent girls, urine collected at Tanner Stage B1 (median age 7.9 years) and Tanner Stage B4 (median age 11.2 years), assessed for menarche every 6 months prior to reaching Tanner stage B4 and every 3 months after reaching Tanner stage B4, Chile	**Late menarche**	**ΣDEHP**	**Child (Tanner Stage B1):** NR	↑
			Child (Tanner Stage B4): NR	↔
		MEHP	**B1**: GM (95% CI): 2.38 (2.13, 2.65) ng/mL (SG-adj)	↑
			B4: 2.23 (1.98, 2.52)	↔
		MEHHP	**B1:** 24.71 (22.22, 27.48)	↑
			B4: 17.33 (15.43, 19.47)	↔
		MEOHP	**B1:** 15.05 (13.56, 16.70)	↑
			B4: 11.21 (10.00, 12.56)	↔
		MECPP	B1: 50.60 (45.91, 55.78)	↔
			B4: 36.00 (32.39, 40.01)	↔

Table 2-16. Summary of Epidemiological Studies of DEHP Exposure and Reproductive Development in Females

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Cathey et al. 2020a, 2020b Cohort, 103 adolescent girls, assessed for reproductive development at age 8–14 years (visit 1) and age 9–18 years (visit 2), Mexico	**Initial breast development (visit 1)**	**ΣDEHP**	NR	↑
		MEHP	Maternal GM: 6.38 (SG-adj)	↔
		MEHHP	23.3	↑
		MEOHP	13.7	↑
		MECPP	41.0	↑
	Progression of breast development (from visit 1 to 2)	ΣDEHP MEHP, MECPP, or MEHHP	See above	↔
		MEOHP	See above	↓
	Pubic hair development or menarche	ΣDEHP, MEHP, MEHHP, MEOHP, or MECPP	See above	↔
	Units were not reported for the GM urinary concentrations.			
Watkins et al. 2014 Cohort, 116 adolescent girls, assessed for reproductive development at age 8–13 years, Mexico	**Pubic hair development**	**MEHP**	Maternal IQR 2.52–9.50 ng/mL	↑
		MEHHP	9.13–37.5	↔
		MEOHP	5.80–24.7	↔
		MECPP	15.1–58.1	↔
	Breast development or menarche	MEHP, MEHHP, MEOHP, MECPP	See above	↔
Wolff et al. 2014 Cohort, 1239 adolescent girls (including 834 normal weight and 405 overweight girls), assessed for reproductive development for 7 years after initial urine collection at age 6–8 years, United States (New York, Ohio, California)	**Pubic hair development (all girls)**	**ΣDEHP**	Child: Interquintile range: 59–510 µg/g Cr	↓
		MEHP	NR	↓
		MEHHP	NR	↓
		MEOHP	NR	↓
		MECPP	NR	↔
	Pubic hair development (normal weight girls)	**ΣDEHP**	See above	↓

Table 2-16. Summary of Epidemiological Studies of DEHP Exposure and Reproductive Development in Females

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
	Pubic hair development (overweight girls)	ΣDEHP	See above	↔
	Breast development or menarche	ΣDEHP, MEHP, MEHHP, MEOHP, MECPP	See above	↔

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; AGD = anogenital distance; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; IQR = interquartile range; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NR = not reported; SG-adj = specific gravity adjusted

The timing of puberty has been examined in four studies using urinary biomarkers of DEHP exposure measured prior to outcome evaluation (e.g., maternal or prepubertal child); results were mixed (Table 2-16). One cohort study reports a delay in breast development in adolescent girls associated with increased prenatal DEHP exposure (Berger et al. 2018), while another reports increased initial breast development but delayed progression of breast development in association with increased prenatal DEHP exposure (Cathey et al. 2020a). In other cohorts, no association was observed between breast development and maternal (Watkins et al. 2014) or prepubertal (Wolff et al. 2014) urinary DEHP metabolite levels. Similarly, increased prenatal DEHP exposure was associated with early pubic hair development in one cohort (Watkins et al. 2014), but not others (Cathey et al. 2020a; Berger et al. 2018). Delayed pubic hair development was associated with increased prepubertal DEHP exposure, particularly in normal weight girls (Wolff et al. 2014). Two studies report an association between prenatal DEHP exposure and increased age at first menarche (Berger et al. 2018); two others did not observe this association (Cathey et al. 2020a; Watkins et al. 2014). Onset of menarche was not associated with prepubertal DEHP exposure (Wolff et al. 2014).

In a cross-sectional study in Taiwanese girls <12 years of age, a positive association between serum FSH and maternal MEEHP and MEOHP levels was observed (Wen et al. 2017). No association was observed for serum testosterone (total or free), LH, estradiol, or SHBG. Cross-sectional studies were not included in Table 2-16; no other data on serum reproductive hormone levels in prepubertal girls were located.

Animal Studies –Female Reproductive Development. Only one study evaluated female reproductive development following inhalation exposure. Ma et al. (2006) reported accelerated vaginal opening and first estrus in weanling female Wistar intermittently exposed to DEHP at concentrations of 0.3–1.6 ppm for 3 or 9 weeks immediately following weaning. Increased serum estradiol and LH were observed at 1.6 ppm following exposure for 3 weeks, and irregular estrous cycles were observed following exposure for 9 weeks. No exposure-related changes in reproductive organ weights were observed. Sexual performance was not evaluated.

In nonhuman primates, exposure to doses ≥500 mg/kg/day for 65 weeks from weaning at 3 months to sexual maturity at 18 months resulted in evidence for accelerated maturation in female marmoset monkeys, including increased serum estradiol, elevated ovary weights, and enlarged corpora lutea (Tomonari et al. 2006).

In Sprague-Dawley rats, significant increases in AGD were observed at PNDs 7 and 21 in female offspring following maternal exposure to doses ≥37.5 mg/kg/day from GD 6 to 21 (lowest dose tested) (Piepenbrink et al. 2005); however, changes in female AGD or AGI at PNDs 21–22 were not observed following gestational and lactational exposure to doses up to 405 mg/kg/day (Grande et al. 2006; Nardelli et al. 2017). In a 2-generation study in Wistar rats, no exposure-related changes were observed in AGD or AGI in F1 or F2 females at doses up to approximately 1,088 mg/kg/day (Schilling et al. 1999, 2001). Similarly, no exposure-related changes in female AGI were observed in offspring of Wistar rats exposed to doses up to 700 mg/kg/day from GD 13 to PND 21 (Venturelli et al. 2019). In CD-1 mice, AGD was not altered following gestational and lactational exposure to doses up to 5 mg/kg/day (Pocar et al. 2012).

In multigenerational studies in rats, delayed vaginal opening was observed in female offspring exposed to doses ≥447 mg/kg/day, but not ≤380 mg/kg/day (Blystone et al. 2010; NTP 2005; Schilling et al. 1999, 2001). Delayed puberty may be due to developmental exposure, peripubertal exposure, or a combination of the two; it may also be secondary to decreased body weights observed at the same doses. However, the percent of littermates with complete vaginal opening on PND 38 was significantly decreased in female offspring of Sprague-Dawley rats exposed to 300 mg/kg/day from GD 8 to PND 21 in the absence of decreased body weight (Nardelli et al. 2017). Similarly, a nonsignificant trend for an approximate 2-day delay in vaginal opening was observed in female offspring of Sprague-Dawley rats exposed to doses ≥135 mg/kg/day from GD 6 to PND 21 in the absence of decreased body weight (Grande et al. 2006). In Wistar rats, vaginal opening was significantly delayed by 3 to 7 days in female offspring (n=31–32/group) following maternal exposure to ≥70 mg/kg/day from GD 13 to PND 21 despite transient elevations in post-weaning offspring body weights (Venturelli et al. 2019). No change in the timing of vaginal opening was observed in Sprague-Dawley rats (n=9–10/group) following direct exposure to doses up to 150 mg/kg/day on PNDs 6–96 (Kim et al. 2018c). The observed differences in vaginal opening may be due to rat strain differences, timing of DEHP exposure, and/or number of animals per dose group.

In contrast, accelerated vaginal opening was observed in Wistar rats following direct exposure to 5 mg/kg/day on PNDs 15–43 (Shao et al. 2019) or 1,000 mg/kg/day on PNDs 22–49 (Liu et al. 2018a). Rats exposed from PND 22 to 49 also showed prolonged estrous and various changes in serum hormone levels (increased progesterone and decreased FSH, LH, and testosterone) at ≥500 mg/kg/day (Liu et al. 2018a). The study authors proposed that precocious puberty was due to early activation of the hypothalamus-pituitary-ovarian axis, as evidenced by elevated serum and/or hypothalamic hormone levels (e.g., growth hormone, gonadotropin-releasing hormone, insulin-like growth factor 1) following exposure to ≥1 mg/kg/day on PNDs 15–43 (Shao et al. 2019) or ≥250 mg/kg/day on PNDs 22–49 (Liu et

al. 2018a). Additional support for early activation of the hypothalamus-pituitary-ovarian axis includes decreased apoptosis and increased neuronal activation in the hypothalamus following exposure to ≥0.2 and 5 mg/kg/day, respectively, on PNDs 15–43 (Shao et al. 2019). Yu et al. (2020) propose that low and high doses of DEHP have a differential effect on the hypothalamus-pituitary-ovarian axis. In support, accelerated vaginal opening and prolonged estrous were observed following exposure to 5 mg/kg/day on PNDs 22–70, but delayed vaginal opening was observed at 500 mg/kg/day. These findings were associated with opposing changes in the hypothalamic kisspeptin system at the low versus high dose. Due to non-monotonic findings, the study by Yu et al. (2020) is not included in the LSE table.

In adult female offspring exposed to DEHP from GD 6 to PND 21, a significant 2-fold increase (over control values) in the number of tertiary atretic ovarian follicles was observed at 405 mg/kg/day; no changes were observed in the numbers of primordial/primary, secondary, or tertiary (healthy) follicles (Grande et al. 2007). A "tendency for dilated interstitial spaces" was reported in the ovaries of female offspring at 405 mg/kg/day (no further details or incidence data provided). No exposure-related changes in the thickness of the uterine or vaginal epithelium were observed. Additionally, no exposure-related changes in estrous cyclicity, serum hormone levels, or reproductive organ weights were observed at maternal doses up to 405 mg/kg/day (Grande et al. 2007). Similarly, no exposure-related changes in serum hormone levels or reproductive organ weight were observed in rat offspring following maternal exposure to doses up to 700 mg/kg/day during gestation and lactation (Nardelli et al. 2017; Venturelli et al. 2019). In a 2-generation study in Wistar rats, no exposure-related changes were observed in female reproductive organ weights in F1 or F2 female weanlings at doses up to approximately 1,088 mg/kg/day (Schilling et al. 2001). In another study, serum estradiol was significantly decreased by >50% in PND 60 female offspring of Sprague-Dawley rat dams exposed to DEHP from GD 14 to PND 0 at doses ≥300 mg/kg/day (Martinez-Arguelles et al. 2011).

In CD-1 mice, ovary weight was significantly elevated by 35–45% in PND 42 offspring at maternal exposure to ≥0.05 mg/kg/day during gestation and lactation (Pocar et al. 2012). When oocytes from female offspring of exposed dams were evaluated for *in vitro* fertilization using unexposed sperm, significantly decreased cleavage and blastocyst rates were observed at maternal doses of 0.05 mg/kg/day; however, this effect was not observed at 5 mg/kg/day (Pocar et al. 2012). The significance of this non-monotonic response is unclear. However, no changes in F1 female fertility were observed at doses up to 500 mg/kg/day in a 1-generation study in C3H/N mice (Schmidt et al. 2012).

In a series of studies with a non-traditional design, female reproductive effects in F1, F2, and/or F3 generation CD-1 offspring were evaluated following exposure to dose ranging from 0.02 to 750 mg/kg/day in F0 dams only from GD 11 until parturition from GD 0.05 to PND 21 (no direct exposure to F1, F2, or F3 mice) (Brehm et al. 2018; Rattan et al. 2017, 2018). Study authors conclude in all studies that there is evidence that DEHP causes generational reproductive effects in females; however, the conclusions are based on numerous non-dose related changes in organ weight, folliculogenesis, estrous cyclicity, and reproductive hormone levels with little concordance between generations and studies. One study (Rattan et al. 2018) reported reduced fertility in F1 offspring at 0.2 mg/kg/day, but not higher doses up to 750 mg/kg/day. A study with a similar design by Pocar et al. (2017) exposed F0 CD-1 mouse dams to DEHP from GD 0.05 to PND 21 and evaluated female reproductive endpoints in F1, F2, and F3 offspring (no direct exposure in F2 or F3 offspring, and F1 exposure only via dam). Fertility was not impacted in any generation, and observed ovarian effects lacked clear dose dependence (e.g., reduced oocyte quality and embryonic developmental competence in all 3 generations at 0.05 mg/kg/day but not 5 mg/kg/day). These findings are consistent with those seen in other studies with similar design. None of the studies provide potential rationales, supporting evidence, or proposed mechanisms of action to explain a non-monotonic dose response. Based on lack of clearly adverse, dose-related findings, these studies were not included in the LSE table.

In a study that evaluated the estrogenic activity of DEHP and other phthalate esters, DEHP induced no reproducible significant increases in uterine wet weight in immature ovariectomized rats following exposure to doses up to 2,000 mg/kg/day for 4 days (Zacharewski et al. 1998).

Mechanisms of Altered Female Reproductive Development. As discussed in Section 2.16 (Mechanisms of Female Reproductive Toxicity), DEHP has been shown to affect mammalian folliculogenesis following exposure during gestation or early life stages (Li et al. 2016; Mu et al. 2015b; Zhang et al. 2013, 2015, 2018c). In addition to interaction with ERs (Cavanagh et al. 2018; Mu et al. 2015b; Zhang et al. 2015), DEHP may alter female reproductive development through interference with estrogen metabolism. Andrade et al. (2006b) observed increased brain aromatase activity in PND 22 female offspring of Sprague-Dawley rats exposed to doses ranging from 0.015 to 405 mg/kg/day during gestation and lactation (Andrade et al. 2006b). As discussed above, altered reproductive development in these female offspring included delayed vaginal opening and increased number of tertiary atretic ovarian follicles at doses ≥15 mg/kg/day (Grande et al. 2006, 2007).

Alterations in ovarian cell proliferation and apoptosis have also been associated with early life exposure to DEHP. Reduced proliferation of pregranulosa precursor cells was observed during the process of primordial folliculogenesis following neonatal exposure via injection (Mu et al. 2015b). Similarly, Li et al. (2016) observed significant increases in the number of apoptotic somatic ovarian cells following early postnatal exposure to DEHP via intraperitoneal injections. Gene expression analysis of ovarian tissue from these animals showed upregulation of mRNA levels of apoptosis and antiproliferation. Li et al. (2016) also observed accumulation of ROS in the ovary and evidence of increased oxidative stress in somatic ovarian cells following *in vitro* exposure. DEHP impaired meiotic progression and repair of DNA damage in fetal mouse oocytes and altered the expression of genes related to apoptosis, gonad development, cell-cell communication, signal transduction, and plasma membrane, extracellular matrix, and ion channel functional classes (Liu et al. 2017).

DEHP may cause heritable epigenetic alterations in germ cells, which may contribute to altered ovarian development (Li et al. 2014; Rattan et al. 2019; Zhang et al. 2013, 2016). Specifically, reduced DNA methylation patterns of genes has been observed in both F1 and F2 offspring oocytes following maternal DEHP exposure to 0.04 mg/kg/day from GD 0.5 to 18.5, including the maternal imprinted genes for insulin like growth factor 2 receptor (*Igf2r*) and paternally expressed 3 (*Peg3*) (Li et al. 2014). Rattan et al. (2019) demonstrated a transgenerational (through the F3 generation) reduction in the expression of ovarian pathways required for folliculogenesis and steroidogenesis following prenatal exposure in mice (exposed from GD 10.5 through birth; gene expression measured on PND 21).

Animal Studies—Other Noncancer (Metabolic Syndrome and Glucose/Insulin Homeostasis). Metabolic syndrome and/or altered glucose homeostasis has been observed in rats and mice following developmental exposure to DEHP during gestation, gestation plus lactation, or lactation.

Adult offspring of Wistar rats exposed to DEHP at doses ≥1 mg/kg/day (lowest dose tested) during gestation showed numerous alterations in glucose homeostasis, including a 16–49% increase in fasting blood glucose, a 21–70% decrease in serum insulin, and a 13–36% decrease in insulin binding protein levels; elevated serum glucose levels were observed in both the glucose and insulin tolerance tests (Rajesh and Balasubramanian 2014). Similarly, adult male offspring of Wistar rats exposed to DEHP at doses ≥10 mg/kg/day (lowest dose tested; female offspring not assessed) during gestation and lactation also showed alterations in glucose homeostasis, including a 36–71% increase in fasting blood glucose, a 100–152% increase in fasting serum insulin, elevated serum glucose levels in both the glucose and insulin tolerance tests, and increased insulin resistance (Rajagopal et al. 2019a). Lactation-only exposure to

DEHP also resulted in altered glucose homeostasis in adult Wistar rat offspring at maternal doses ≥1 mg/kg/day (Mangala Priya et al. 2014; Venturelli et al. 2015). Observed effects included elevated fasting blood glucose, decreased insulin sensitivity, and decreased *ex vivo* insulin secretion by isolated pancreatic islet cells. In contrast, evidence of altered glucose homeostasis in adult Wistar rat offspring following gestation plus lactation exposure was not observed until much higher maternal doses of DEHP (Venturelli et al. 2019). Observed effects included decreased *ex vivo* insulin secretion by isolated pancreatic islet cells at ≥70 mg/kg/day and elevated fasting blood glucose levels at 700 mg/kg/day. Elevated fasting blood glucose was also observed in male PND 22 Wistar rat offspring (female offspring were not assessed) following lactation-only exposure to DEHP at a maternal dose of 100 mg/kg/day, but not ≤10 mg/kg/day (Parsanathan et al. 2019). With early postweaning exposure on PNDs 22–52, elevated fasting blood glucose was observed in PND 53 male Wistar rats at 75 mg/kg/day, but not 7.5 mg/kg/day (Venturelli et al. 2015).

Altered glucose homeostasis, along with pancreatic dysfunction, was also observed in weanling and adult offspring of Wistar rats following maternal exposure to doses ≥1.25 mg/kg/day during gestation and lactation (Lin et al. 2011). Effects observed at weaning included decreased fasting blood glucose and serum insulin levels, and lower blood glucose levels and insulin secretion in glucose and insulin tolerance testing at both exposure levels. By PNW 15, blood glucose levels were comparable among all groups, and serum insulin levels were elevated in female offspring only. No significant differences were observed in glucose levels in females during the glucose challenge test; however, elevated insulin levels were persistent. In exposed males, enhanced glucose tolerance was observed. However, at PNW 27, exposure-related changes in female offspring resumed, including elevated fasting blood glucose and decreased serum insulin; significantly elevated glucose levels and significantly reduced insulin levels were also observed with glucose tolerance tests. In male offspring, no changes were observed in blood glucose, but serum insulin levels were elevated and greater insulin levels were required for glucose clearance. No exposure-related changes in fasting glucagon levels were observed at any time point. In insulin tolerance tests, glucose lowering effects were increased in all exposed groups at PNW 3, but results were comparable to controls at PNWs 15 and 27. In the pancreas, decreased β-cell mass and pancreatic insulin content were observed in exposed offspring at PND 21, but there were no significant changes in pancreas weight or β-cell area. At PNW 17, pancreatic weights were elevated in female offspring, but β-cell area and mass and pancreatic insulin content were decreased. In DEHP-exposed male offspring, β-cell area was increased and a trend toward increased mass was observed; pancreatic weight and insulin content were comparable to controls. With glucose-stimulation, islets from exposed female offspring had lower insulin secretion compared with controls. In this study, no changes in

maternal serum insulin or blood glucose levels were observed at doses up to 6.25 mg/kg/day (Lin et al. 2011), indicating that developing offspring may be more susceptible to pancreatic toxicity than adult animals.

Evidence of metabolic syndrome has been reported in mice following gestational exposure to DEHP. In ICR mouse offspring exposed to a maternal dose of 0.2 mg/kg/day, observed effects at PNW 12 included altered glucose homeostasis (glucose and insulin tolerance tests), increase in serum lipid levels, reduced energy expenditure, and white adipocyte hypertrophy and increased lipid deposits in the liver cells (Fan et al. 2020). In C57Bl/6J mouse offspring exposed to a maternal dose of 0.05 mg/kg/day, observed effect at PNW 9 included increased visceral (gonadal) fat pad weight and increased serum leptin, insulin, triglycerides, total cholesterol, and fasting glucose levels (Gu et al. 2016). However, in a study with gestation plus lactation exposure in C57BL/6J x FVB mice, no exposure-related changes in glucose or insulin tolerance were observed in PNWs 30–31 mice following maternal exposure to 33 mg/kg/day from 2 weeks premating through lactation (Bastos Sales et al. 2018). No changes were observed in the sucrose preference test at PNW 40. Following exposure to a high-fat diet for 9 weeks, no DEHP-related changes in serum insulin, glucagon, or fasting glucose levels were observed at PNWs 55–57.

Insulin sensitivity was observed in PNW 16 FVB mouse offspring following maternal exposure to 500 mg/kg/day throughout gestation and lactation followed by high-fat diet consumption for 13 weeks (Hunt et al. 2017). Following injection with insulin, all DEHP-exposed wild-type mice became lethargic and 5/6 entered hypoglycemic shock. All high-fat diet control animals were insulin tolerant. Insulin sensitivity was dependent on PCNA, as both control and DEHP-exposed transgenic mice without functional PCNA were insulin tolerant. No changes in glucose tolerance at PNW 15 were observed in control or exposed mice of either genotype. Due to use of a high-fat diet and use of only one high-dose exposure group, this study was not included in the LSE table. However, this study suggests that DEHP-induced changes in insulin tolerance may be mediated via PCNA.

Mechanisms of Developmental Metabolic Syndrome and Altered Glucose/Insulin Homeostasis). Several tissues have shown decreased glucose uptake and oxidation, decreased insulin binding, and/or decreased glycogen content following developmental exposure to DEHP, including skeletal muscle, liver, and cardiac tissue (Mangala Priya et al. 2014; Parsanathan et al. 2019; Rajagopal et al. 2019a; Rajesh and Balasubramanian 2014).

Several genes or gene products involved in insulin signaling were dysregulated in adult rat offspring following developmental exposure to DEHP. These include downregulation, posttranslational modification, and/or epigenetic silencing of glucose transporters (*GLU2*, *GLU4*) and insulin receptors (IRβ) (Rajagopal et al. 2019a, 2019b; Rajesh and Balasubramanian 2014). Additionally, alterations in transcription factors involved in glycogenesis and gluconeogenesis were observed in the liver of developmentally exposed rats (Rajagopal et al. 2019a, 2019b).

Alterations in mRNA expression of genes essential for pancreatic β-cell function were also observed following developmental exposure to DEHP, including downregulation of *Pdx-1* and upregulation of *Atf4*, *Atf6*, *Bip*, and *Ucp2* (Lin et al. 2011; Venturelli et al. 2019). DEHP also produced cytotoxicity in cultured pancreatic β cells (INS-1) and the apoptotic process was shown to be mediated by oxidative stress and autophagy (Li et al. 2019a; She et al. 2017).

Animal Studies—Other Developmental Effects. Other animal studies have evaluated development and function of the lungs, cardiovascular system, endocrine glands (adrenal, pituitary, thyroid), and immune system following developmental DEHP exposure (Chen et al. 2010; Christiansen et al. 2010; Dong et al. 2019; Kobayashi et al. 2006; Martinez-Arguelles et al. 2011, 2013; Piepenbrink et al. 2005; Wei et al. 2012), but data are too limited to draw conclusions. These studies are discussed in Sections 2.4 (Respiratory), 2.5 (Cardiovascular), 2.13 (Endocrine), and 2.14 (Immunological), respectively.

Summary. Human and animal data indicate that the developing male reproductive system is a sensitive target of DEHP toxicity. In a systematic review, NAS (2017) concluded that DEHP is presumed to be a reproductive hazard to humans based on evidence integration of the animal and the human evidence on DEHP and effects on AGD and fetal testosterone and is suspected to be a reproductive hazard to humans based on evidence integration of the animal evidence and the human evidence on DEHP and fetal hypospadias. Data for early puberty and delayed mental and psychomotor development in humans following early life DEHP exposure are mixed. Additional animal studies report some evidence that DEHP exposure can also adversely affect the developing female reproductive system as well as the nervous, hepatic, and renal systems following DEHP exposure prior to sexual maturity. Altered glucose homeostasis and metabolic syndrome have also been reported following developmental exposure. Fetotoxic and teratogenic effects have been observed at higher exposure levels following gestational exposure in animals.

2.18 OTHER NONCANCER

Epidemiology Studies. Several cross-sectional studies in adults that used urinary metabolite levels to assess DEHP exposure (Table 2-17) have reported associations with increased fasting blood glucose, increased serum insulin, and/or insulin resistance (as assessed by homeostatic model assessment-insulin resistance [HOMA-IR]) (Attina and Trasande 2015; Chen et al. 2017; Dales et al. 2018; Huang et al. 2014b; James-Todd et al. 2012; Li et al. 2019a; Lin et al. 2016, 2020; Trasande et al. 2013b). Dales et al. (2018) also reported an association between DEHP exposure and increases levels of HbA1c. In addition, a panel study in Korea with repeated same-day urine and blood samples showed associations between increased fasting serum glucose (Kim et al. 2013) or insulin resistance (Kim and Hong 2014; Kim et al. 2013) and higher levels of DEHP metabolites in urine. In contrast, a small number of studies did not observe associations between DEHP exposure and measures of glucose homeostasis (Ko et al. 2019; Stahlhut et al. 2007) A study in obese subjects (Dirinck et al. 2015) yielded conflicting results, as there was a relationship between decreased insulin sensitivity and DEHP metabolite levels and associations between decreased insulin resistance and DEHP metabolite levels.

Findings pertaining to glucose homeostasis in children and adolescents are inconsistent (Table 2-17). Han et al. (2019) reported an association between insulin resistance and urinary DEHP metabolite levels at 7–9 years of age, but not 3–5 years; conversely, fasting blood glucose levels were associated with increased urinary DEHP metabolite levels at 3–5 years of age, but not 7–9 years. Kim et al. (2018a) observed a positive association between urinary MEHHP levels in prepubertal girls and insulin resistance; this was not observed with other metabolites or in pubertal girls. In other cross-sectional studies, no association between insulin resistance and/or fasting blood glucose and DEHP metabolite levels in urine were observed in children aged 8–14 years (Watkins et al. 2016) or adolescents aged 12–19 years (Chen et al. 2017).

Disparate findings in the cross-sectional studies may reflect differing susceptibilities across populations, genders, or ages, or differences in the covariates considered in the studies. Additionally, due to the cross-sectional design, it is not possible to determine if reported changes in glucose homeostasis in some studies are acute reactions to exposure or represent a trend toward increased blood glucose following chronic exposure to DEHP. Finally, cross-sectional studies may also be vulnerable to spurious findings due to reverse causality if higher urinary metabolite levels occur as a consequence of higher exposure via medications or personal care products in persons with impaired glucose homeostasis. However, the finding of increased risk of impaired glucose homeostasis is supported by a case-control study nested

Table 2-17. Selected Epidemiological Studies of DEHP Exposure and Diabetes or Glucose Homeostasis

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Attina and Trasande 2015 Cross-sectional, 356 adolescents (age 12–19 years), United States (NHANES)	**Insulin resistance (HOMA-IR >4.39)**	**ΣDEHP**	IQR: 0.07–0.32 µM	↑
		MEHP	NR	↑
		MEHHP	NR	↑
		MEOHP	NR	↑
		MECPP	NR	↑
	HOMA-IR	ΣDEHP	See above	↔
Chen et al. 2017 Cross-sectional, 786 adolescents and young adults (234 adolescents age 12–19 years, 552 young adults age 20–30 years), Taiwan	**Serum insulin or HOMA-IR (young adults)**	**MEHP**	All subjects: mean (SD): 5.05 (12.86) µg/g Cr	↑
		MEHHP	26.70 (2.53)	↔
		MEOHP	16.65 (2.51)	↔
	Fasting glucose (young adults)	MEHP, MEHHP, or MEOHP	See above	↔
	Serum insulin, HOMA-IR, or fasting glucose (adolescents)	MEHP, MEHHP, or MEOHP	See above	↔
Dales et al. 2018 Cross-sectional, 2,119 non-diabetic adolescents and adults (age 12–79 years, mean age 37.28 years), Canada	**HbA1c (%)**	**ΣDEHP**	Mean (SE): 47.28 (1.45) µg/L	↑
		MEHP	2.09 (0.05)	↑
		MEHHP	12.72 (0.32)	↑
		MEOHP	7.53 (0.19)	↔
	HOMA-IR	**ΣDEHP, MEHHP, or MEOHP**	See above	↑
		MEHP	See above	↔
	HOMA-β (%)	**ΣDEHP, MEHP, MEHHP, or MEOHP**	See above	↑
	Fasting glucose	**ΣDEHP or MEHHP**	See above	↑
		MEHP or MEOHP	See above	↔

Table 2-17. Selected Epidemiological Studies of DEHP Exposure and Diabetes or Glucose Homeostasis

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
	Fasting insulin	**ΣDEHP**	See above	↑
		MEHP, MEHHP, or MEOHP	See above	↔
Dirinck et al. 2015 Cross-sectional, 123 adult obese subjects without a history of type 2 diabetes, Belgium	**Insulin resistance**	MEHP	Range: 0.49–181.9 µg/g Cr	↔
		MEHHP	2.6–135.8	↓
		MEOHP	0.82–42.3	↑
		MECPP	0.1–268.8	↔
	AUC insulin	MEHP, MEHHP, or MECPP	See above	↔
		MEOHP	See above	↑
	Insulin sensitivity	MEHP, MEHHP, or MECPP	See above	↔
		MEOHP	See above	↓
	HbA1c levels, AUC glucose, HOMA-IR, or insulinogenic index	MEHP, MEHHP, MEOHP, or MECPP	See above	↔
Han et al. 2019 Cross-sectional, 164 children assessed at age 3–5 and 7–9 years, South Korea	**HOMA-IR**	**ΣDEHP**	3–5 years: IQR: 258.18–595.69 µg/g Cr	↔
			7–9 years: 159.43–370.86	↑
		MEHP	3–5 years**:** 14.14–37.55	↔
			7–9 years: 10.35–31.76	↑
		MEHHP	3–5 years: 89.79–212.80	↔
			7–9 years: 58.19–127.45	↑
		MEOHP	3–5 years: 54.92–134.51	↔
			7–9 years: 33.33–74.17	↑
		MECPP	3–5 years: 75.08–190.57	↔
			7–9 years: 49.22–120.65	↑

Table 2-17. Selected Epidemiological Studies of DEHP Exposure and Diabetes or Glucose Homeostasis

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
	Fasting glucose	ΣDEHP, MEHP, MEOHP, or MECPP	3–5 years: see above	↔
			7–9 years: see above	↔
		MEHHP	**3–5 years:** see above	↑
			7–9 years: see above	↔
Huang et al. 2014b Cross-sectional, 3,083 non-diabetic, nonpregnant subjects (age 12–80 years), United States (NHANES)	**Fasting blood glucose or HOMA-IR**	**ΣDEHP** (MEHP, MEHHP, MEOHP)	Men: IQR: 5.3–19.7 µmol/100 g Cr Women: 6.5–23.1	↑
James-Todd et al. 2018 Cohort, 245 pregnant women without history of diabetes (mean age 35.3 years), United States (Massachusetts)	Non-fasting blood glucose (GW 27)	ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	1st trimester (median GW 7): IQR: 0.09–0.36 nmol/mL (SG-adj)	↔
			2nd trimester (median GW 21): 0.07–0.23	↔
James-Todd et al. 2016b Cohort, 298 pregnant women with full term births (47 with impaired glucose tolerance), mean age 31.9 years, United States (Massachusetts)	**Impaired glucose tolerance**	**ΣDEHP** (MEHP, MEHHP, MEOHP, MECPP)	Controls: GM: 0.2–0.8 µmol/L (SG-adj) IGT cases: 0.2–1.4	↑
	Blood glucose	ΣDEHP	See above	↔
James-Todd et al. 2012 Cross-sectional, 215 female cases of self-reported diabetes, 2,135 women without diabetes (age 20–79 years), United States (NHANES)	Fasting blood glucose or A1c%	ΣDEHP (MEHP, MEHHP, MEOHP)	GM (95% CI): 1,110 (1,030, 1,200) (units not reported)	↔
	HOMA-IR	**ΣDEHP**	See above	↑

Table 2-17. Selected Epidemiological Studies of DEHP Exposure and Diabetes or Glucose Homeostasis

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Kim et al. 2018a Cross-sectional, 137 girls including 68 prepubertal and 69 pubertal (age 6–13 years), Korea	**HOMA-IR (all prepubertal girls)**	MEHP	All girls: IQR: 9.7–19.6 µg/g Cr	↔
		MEHHP	26.8–56.7	↑
		MEOHP	21.1–45.1	↔
		MECPP	55.5–140	↔
	HOMA-IR (all pubertal girls)	MEHP, MEHHP, MEOHP, or MECPP	See above	NR
	Pubertal girls were classified as girls who had reached Tanner stage 2 of breast development at the time of examination.			
Kim and Hong 2014; Kim et al. 2013 Panel study, 560 subjects (146 men, 414 women; age 60–87 years), Korea	**HOMA-IR (both sexes; women); fasting serum glucose (both sexes)**	**ΣDEHP**	NR	↑
		MEHHP	Range: 1.71–317.26 ng/mL	NR
		MEOHP	0.212–231.44	NR
	Fasting serum glucose (women)	ΣDEHP	See above	↔
Ko et al. 2019 Cross-sectional, 435 adults (mean age 32.16 years), Taiwan	High HOMA-IR (>75th percentile) or high fasting blood glucose (≥100 mg/dL)	ΣDEHP	NR	↔
		MEHP	25th–95th percentile: 0.269–2.789 µg/g Cr	NR
		MEHHP	0.908–6.045	NR
		MEOHP	0.486–2.603	NR
Li et al. 2019a, 2019b Cross-sectional, 1,605 adults (mean age 37.4 years), United States (NHANES)	**HOMA-IR**	**ΣDEHP**	IQR: 0.12–0.58 µmol/L	↑
		MEHP	0.9–5.7 ng/mL	NR
		MEHHP	10.5–52.8	NR
		MEOHP	7.0–35.9	NR
		MECPP	17.0–78.8	NR
Lin et al. 2020 Cross-sectional, 792 adolescents and adults (age 12–30 years; mean age 21.3 years), Taiwan	**HOMA-IR**	**MEHP**	GM (95% CI): 6.1 (5.1, 7.3) µg/g Cr	↑
		MEHHP	27.9 (26.1, 30.0)	↔
		MEOHP	17.5 (16.4, 18.5)	↔

Table 2-17. Selected Epidemiological Studies of DEHP Exposure and Diabetes or Glucose Homeostasis

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Lin et al. 2016 Cross-sectional, 793 students, 303 with and 486 without elevated blood pressure in childhood (mean age 21.28 years), Taiwan	**HOMA-IR**	**MEHP**	GM (95% CI): 6.1 (5.1, 7.32) µg/g Cr	↑
		MEHHP	27.90 (26.05, 29.96)	↔
		MEOHP	17.48 (16.44, 18.54)	↔
Robledo et al. 2015 Cohort, 72 pregnant women (age 18–38 years) without diabetes, United States (Oklahoma)	Blood glucose	ΣDEHP	IQR: 36.82–126.00 ng/mL	↔
		MEHP	1.40–7.75	↔
		MEHHP	10.35–40.85	↔
		MEOHP	7.70–24.20	↔
		MECPP	16.90–54.20	↔
Shapiro et al. 2015 Cohort, 1,274 pregnant women (age >18 years), 47 cases of impaired glucose tolerance (IGT) and 43 cases of gestational diabetes mellitus (GDM)	GDM or IGT	ΣDEHP	NR	↔
		MEHP	Controls: GM (GSD): 2.6 (2.5) ng/mL (SG-adj) IGT cases: 2.3 (2.4) GDM cases: 2.7 (2.9)	NR
		MEHHP	Controls: 10.6 (2.5) IGT cases: 10.4 (2.4) GDM cases: 11.4 (3.0)	NR
		MEOHP	Controls: 7.4 (2.3) IGT cases: 6.9 (2.2) GDM cases: 7.8 (2.7)	NR
Stahlhut et al. 2007 Cross-sectional, 1,451 adult males (age >18 years), United States (NHANES)	HOMA-IR	MEHP	Mean (SE): 11 (1.3) µg/g Cr	↔
		MEHHP	65.8 (7.9)	↔
		MEOHP	38.7 (4.5)	↔

Table 2-17. Selected Epidemiological Studies of DEHP Exposure and Diabetes or Glucose Homeostasis

Reference, study type, and population	Outcome evaluated	Metabolite	Urine concentration	Result
Sun et al. 2014b Cohort, 394 females with type-2 diabetes and 393 controls (NHS cohort; age 23–79 years) and 577 females with type-2 diabetes and 577 controls (NHSII cohort; age 32–52 years), United States	**Type 2 diabetes**	**ΣDEHP**	NHS cases: IQR: 154.4–545.8 nmol/L NHS controls: 142.8–463.7 NHSII cases: 201.4–586.3 NHSII controls: 170.8–522.3	NR
		MEHP	NR	↔
		MEHHP	NR	↔
		MEOHP	NR	↔
		MECPP	NR	↑
Trasande et al. 2013b Cross-sectional, 766 adolescents (age 12–19 years), United States (NHANES)	**HOMA-IR (>2 SD above mean)**	**ΣDEHP**	IQR: 0.17–0.71 µM	↑
		MEHP	NR	↔
		MEHHP	NR	↑
		MEOHP	NR	↑
		MECPP	NR	↑
Watkins et al. 2016 Cross-sectional, 250 children (age 8–14 years), Mexico	Fasting serum glucose	ΣDEHP	IQR: 3.09–10.3 µmol/L	↔

[a]Metabolite units and reporting (range, IQR, etc.) defined for the first metabolite per study are applicable to all subsequent metabolites for that study unless otherwise noted.

Bold font indicates association with DEHP or DEHP metabolites: ↑ = association with increase; ↓ = association with decrease; ↔ = no association

ΣDEHP = sum DEHP metabolites; AUC = area under the curve; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; GSD = geometric standard deviation; GW = gestation week; HbA1c = glycosylated hemoglobin; HOMA-β = homeostatic model assessment-beta cell function; HOMA-IR = homeostatic model assessment-insulin resistance; IQR = interquartile range; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; NHANES = National Health and Nutrition Examination Survey; NHS = Nurses' Health Study; NR = not reported; SD = standard deviation; SE = standard error; SG-adj = specific gravity-adjusted

within the Nurses' Health Study and Nurses' Health Study II (Sun et al. 2014b) that examined incident diabetes and thus, was not confounded by reverse causality. In this study, a pooled analysis of the two nurses' studies showed increased odds of developing type 2 diabetes with increased levels of MECPP in urine. No association was observed between type 2 diabetes and urinary levels of other DEHP metabolites or the sum of all DEHP metabolites.

Little information was located on the association between DEHP exposure and gestational diabetes (Table 2-17). In two cohort studies (Robledo et al. 2015; Shapiro et al. 2015), no association between DEHP exposure and impaired glucose tolerance or gestational diabetes was observed (Table 2-17). A third cohort study reported reduced odds of having impaired glucose tolerance during pregnancy with increased DEHP concentration in maternal urine (James-Todd et al. 2016b). However, blood glucose levels were not associated with prenatal DEHP exposure (James-Todd et al. 2016b, 2018).

Animal Studies. Glucose homeostasis may be impaired in animals following exposure to DEHP. In rats, evidence of altered glucose metabolism and homeostasis was observed following intermediate-duration exposure to doses ≥5 mg/kg/day (Aydemir et al. 2018; Rajesh et al. 2013; Xu et al. 2018; Zhang et al. 2017). Altered endpoints included elevated serum glucose levels, decreased glycogen levels and glucose uptake in visceral adipose tissue, and/or elevated serum glucose and insulin levels during glucose and insulin tolerance tests. However, other studies in rats reported no changes in serum glucose following exposure to gavage doses up to 10,000 mg/kg/day for 4 weeks or 1,000 mg/kg/day for 9 weeks (Dalgaard et al. 2000). In a 13-week study, increased serum glucose was observed in male rats exposed to doses ≥850.1 mg/kg/day; this effect was not observed in males at doses ≤261.2 mg/kg/day or females at doses up to 1,857.6 mg/kg/day (Myers 1992b). In mice, evidence for impaired glucose homeostasis (e.g., elevated fasting blood glucose, elevated glucose levels after glucose challenge) was observed after acute exposure to 2,000 mg/kg/day or intermediate-duration exposure to ≥180 mg/kg/day (Ding et al. 2019; Lee et al. 2019a; Li et al. 2018). However, serum glucose changes were not observed in B6C3F1 mice exposed to doses up to 7,899 mg/kg/day for 28 days (Myers 1992a).

Several developmental studies have also reported altered glucose homeostasis and impaired pancreatic β-cell function in rats following prenatal and/or early postnatal exposure to oral doses of 1–10 mg/kg/day (e.g., Lin et al. 2011; Mangala Priya et al. 2014; Rajesh and Balasubramanian 2014). In these studies, no changes in maternal serum insulin or blood glucose levels were observed at doses up to 6.25 mg/kg/day, indicating that developing offspring may be more susceptible to pancreatic toxicity (Lin et al. 2011). See Section 2.17 (Developmental) for more details on these studies.

There is limited evidence of metabolic syndrome in laboratory animals following oral exposure to DEHP. Increased volume and number of adipose cells in unspecified adipose tissue were observed in rats following gavage exposure to ≥5 or 500 mg/kg/day, respectively, for 8 weeks (Zhang et al. 2019, 2020c). In another rat study with the same exposure paradigm, irregular adipocytes and macrophage infiltration in adipose tissue was observed at ≥50 mg/kg/day with increased number and volume of adipocytes at 500 mg/kg/day (Zhou et al. 2019). Zhou et al. (2019) reported increased serum leptin (an appetite-controlling hormone) and decreased serum adiponectin (regulates lipid and glucose metabolism) at ≥50 mg/kg/day; these parameters were unchanged in the study by Zhang et al. (2019, 2020c). Serum leptin was also elevated in rats exposed to ≥50 mg/kg/day for 28 days (Xu et al. 2018). In mice, increases in visceral adipose tissue and adipocyte hypertrophy were observed following exposure to dietary doses ≥0.05 mg/kg/day for 8 weeks; this finding was accompanied by significant increases in body weight and food intake (Schmidt et al. 2012). Significant increases in leptin were also observed at 500 mg/kg/day. Similarly, significant increases in visceral adipose tissue were observed in F0 mouse dams exposed to dietary doses ≥0.05 mg/kg/day from 1 week premating through PND 21 (Schmidt et al. 2012). Visceral adipose tissue was also elevated in F1 adult female offspring at maternal doses ≥0.05 mg/kg/day (Schmidt et al. 2012). No changes in retroperitoneal or ovarian adipose tissue weights were observed in Wistar rat dams exposed to DEHP at doses up to 700 mg/kg/day from GD 13 to PND 21 (Venturelli et al. 2019). Rajesh and Balasubramanian (2014) also reported significant increases in adipose tissue in adult rat offspring following maternal exposure to ≥1 mg/kg/day via gavage from GD 9 to 21 (Rajesh and Balasubramanian 2014). However, a significant *decrease* in adipose tissue was reported in PND 42 female mouse offspring at maternal dietary doses ≥0.05 mg/kg/day from GD 0 to PND 21 (Pocar et al. 2012) and in PND 21 rat offspring following maternal gavage exposure to ≥1.25 mg/kg/day from GD 9 to 21 (Lin et al. 2011).

Extensive fur loss was reported in rats exposed to dietary DEHP at doses ≥1,414 mg/kg/day for 17 weeks (Gray et al. 1977). Rats showing fur loss were also described as “emaciated” by study authors, with decreases in food consumption and body weight of >25%. Therefore, it is unclear if fur loss is a primary health effect or secondary to overall poor health.

Mechanisms of Impaired Glucose Homeostasis and Metabolic Syndrome. Several tissues have shown decreased glucose uptake and oxidation, decreased glycogen content, an/or alterations in metabolic pathways involved in glucose metabolism following exposure to DEHP, including cardiac, liver, and adipose tissue (Ding et al. 2019; Li et al. 2018; Rajesh et al. 2013). Additionally, several genes or gene

products involved in glucose transport, insulin signaling, and lipid metabolism were dysregulated in rodents following exposure to DEHP (Ding et al. 2019; Rajesh et al. 2013; Xu et al. 2018; Zhang et al. 2017, 2019, 2020c). Wang et al. (2020) propose that significant alterations in the gut microbiome following oral DEHP exposure may contribute to increased risk of diabetes. Following a 30-day oral exposure, Sprague-Dawley rats, showed an increase in bacterial species *Fimicutes* and *Proteobacteria*, which are associated with obesity and diabetes. Sprague-Dawley rats also showed DEHP-associated weight gain. In contrast, Wistar rats and BALB/c and C57BL/6J mice did not have increased *Fimicutes* and *Proteobacteria* and showed normal weight gain.

Proposed mechanisms for impaired glucose homeostasis in developing animals are discussed in Section 2.17 (Developmental).

Summary. Several epidemiological studies, primarily cross-sectional, found potential associations between DEHP exposure and diabetes-related outcomes (e.g., impaired glucose homeostasis) in humans. A limited number of animal studies report altered glucose homeostasis and metabolic syndrome.

2.19 CANCER

Epidemiological Studies—Cancer. One population-based study did not find an association between DEHP exposure and breast cancer using NHANES data for urinary MEHP, MEHHP, or MEOHP collected during the 2003–2010 annual survey cycles (Morgan et al. 2017). Additional epidemiological studies of the association between cancer and DEHP exposure in humans are limited to case-control studies in which exposure (as urinary metabolite levels) was measured after the outcome (cancer) was observed. Cancers evaluated in these studies include breast cancer (Holmes et al. 2014; Lopez-Carrillo et al. 2010; Martinez-Nava et al. 2013; Merida-Ortega et al. 2016; Reeves et al. 2019), prostate cancer (Chuang et al. 2020), and thyroid cancer (Liu et al. 2020; Marotta et al. 2019; Miao et al. 2020). There is no information (qualitative or quantitative) on exposures prior to incidence/diagnosis that could have been involved in tumor induction. Furthermore, cancer treatments could increase exposure to, and excretion of, phthalates from medical equipment. Thus, these studies are not useful for evaluating the carcinogenicity of DEHP.

Animal Studies—Cancer. Lifetime exposure of hamsters to 0.001 ppm DEHP did not result in any significant increase in the incidence of tumors (Schmezer et al. 1988). Because the concentration in this

study was very low, it is not possible to reach conclusions concerning whether or not higher concentrations might produce different results.

Hepatic Cancer. Several chronic exposure studies in rodents indicate that DEHP can cause liver tumors in rats and mice. Hepatocellular adenomas and carcinomas have consistently been reported following chronic oral exposure in F344 rats at doses ≥394 mg/kg/day (Cattley et al. 1987; David et al. 1999, 2000a; Hayashi et al. 1994; Kluwe et al. 1982a, 1982b, 1985; NTP 1982; Rao et al. 1987, 1990) and in B6C3F1 mice at doses ≥354.2 mg/kg/day (David et al. 1999, 2000b; Kluwe et al. 1982a, 1982b, 1985; NTP 1982). Only David et al. (1999, 2000a) reported an increased incidence of hepatocellular tumors in male F344 rats at lower doses, observing a dose-related increase in tumors at dietary doses ≥147 mg/kg/day, but not ≤29 mg/kg/day (David et al. 1999, 2000a). NTP (1982) reported metastasis of hepatic carcinoma to the lungs in 37% of tumor cases in DEHP-exposed mice; metastasis was not observed in control mice or exposed or control rats.

A nonsignificant increase in hepatocellular adenomas and carcinomas was observed in male Sprague-Dawley rats following lifetime exposure to 300 mg/kg/day (Voss et al. 2005). In contrast, Ganning et al. (1991) did not observe any liver tumors in male Sprague-Dawley rats following exposure to doses up 1,400 mg/kg/day for 102 weeks; however, 7–18 animals were included in each dose group. In Sherman rats, hepatocellular tumors were not significantly increased following chronic exposure to DEHP, but the maximum tested dose was only 200 mg/kg/day (Carpenter et al. 1953). In other species, liver tumors were not elevated following 1-year exposure of dogs at doses up to 56.6 mg/kg/day or guinea pigs at doses up to 64 mg/kg/day (Carpenter et al. 1953). Due to study design deficiencies (low animal number and/or low doses), it is unclear if the studies by Ganning et al. (1991) or Carpenter et al. (1953) were adequate to assess potential carcinogenicity.

Hepatocellular adenomas were also observed in 1/15, 2/15, and 4/15 male rasH2 transgenic mice following exposure to DEHP at a dietary dose of 1, 100, or 1,100 mg/kg/day, respectively, for 26 weeks, compared to 0/15 controls (Toyosawa et al. 2001). RasH2 transgenic mice carry a human prototype c-Ha-ras gene. Due to increased susceptibility to developing cancer, it is proposed that carcinogenic potential can be assessed using shorter-durations and fewer animals than a standard 2-year bioassays. Hepatocellular tumors were not induced in similarly treated female rasH2 transgenic mice or male or female wild-type mice (Toyosawa et al. 2001).

Mechanisms of Hepatic Cancer. The mechanistic events associated with DEHP liver toxicity are described briefly in Section 2.9 (Mechanisms of Liver Toxicity). The exact mechanism(s) by which DEHP induces hepatic cancer in rodents are not precisely known; however, the available data suggest that multiple molecular targets and pathways are affected in multiple liver cell types (Guyton et al. 2009; Ito et al. 2019; Melnick 2001; Rusyn and Corton 2012).

As discussed in Section 2.9, DEHP activates PPARα in rats and mice (Rusyn and Corton 2012). Therefore, it follows that observed liver tumors in rodents may be PPARα-dependent. Key events identified in this mode of action are: (1) PPARα activation; (2) alterations in cell growth pathways; (3) perturbation of hepatocyte growth and survival; (4) selective clonal expansion of preneoplastic foci cells; and (5) increases in hepatocellular adenomas and carcinomas (apical event) (Corton et al. 2018). Isenberg et al. (2000, 2001) proposed that increased peroxisomal proliferation, increased replicative DNA synthesis, and inhibition of GJIC observed in rat and mouse livers following oral exposure to DEHP may contribute to PPARα-dependent hepatic tumor formation. Observed losses in GJIC following oral exposure to DEHP may permit unchecked proliferation of transformed cells. Inhibition of GJIC was not observed in exposed hamsters, a species that is refractory to PPARα-dependent tumors (Isenberg et al. 2000).

It is generally accepted that the PPARα mode of action is not relevant to humans due to differences observed in key events downstream of PPARα activation (Corton et al. 2018; Klaunig et al. 2003; Maloney and Waxman 1999). Guyton et al. (2009) reported that PPARα activation may not be essential to rodent liver tumor formation since liver tumors have been observed in some studies using PPARα-null mice; however, the validity of this argument has been questioned by Corton et al. (2018). Concerns regarding conclusions reached by Guyton et al. (2009) include: (1) all liver tumor types, including hepatoblastomas, which originate from a different cell population compared with adenomas and carcinomas, were combined for statistical analysis; (2) use of DEHP doses that did not cause liver tumors in wild-type mice in studies reporting tumors in PPARα-null mice; (3) comparison of findings in PPARα-null mice to non-concurrent controls of a different strain; and (4) different molecular environments in PPARα-null mice compared with wild-type mice (e.g., increased levels of background and DEHP-inducible levels of oxidative stress).

Other molecular targets possibly related to DEHP-induced liver cancer include activation of nuclear factor kappa B (NFκB) leading to chronic inflammation or CAR activation resulting in cell proliferation and foci formation (Ito et al. 2019; Wei et al. 2017).

The genotoxicity data for DEHP are presented in Section 2.20. DEHP has been shown to induce DNA damage, chromosomal effects, and cell transformation (Caldwell 2012).

Endocrine Cancer. There is limited evidence of pancreatic adenomas following chronic exposure to DEHP; however, these tumors have only been observed in male F344 rats at high dose levels (789–1,600 mg/kg/day). Pancreatic acinar cell adenomas were reported in male F344 rats following chronic exposure to 789 mg/kg/day; incidences were not increased at doses ≥147 mg/kg/day in males or at doses up to 939 mg/kg/day in females (David et al. 2000a). Rao et al. (1990) also reported an increased incidence of pancreatic islet cell adenomas in male F344 rats exposed to 1,600 mg/kg/day for 108 weeks. Pancreatic tumors were not elevated in another chronic-duration study in F344 rats; however, the maximal tested dose in male F344 rats was 674 mg/kg/day (Kluwe et al. 1982a, 1982b, 1985; NTP 1982). In other species, pancreatic tumors were not elevated compared to controls following chronic exposure in dogs at doses up to 56.6 mg/kg/day (Carpenter et al. 1953), guinea pigs at doses up to 64 mg/kg/day (Carpenter et al. 1953), or mice at doses up to 1,821 mg/kg/day (David et al. 1999, 2000b; Kluwe et al. 1982a, 1982b, 1985; NTP 1982).

Reproductive Cancer. One study reported an increased incidence of Leydig cell tumors in male rats following chronic oral exposure to DEHP. Voss et al. (2005) reported a significant increase in the incidence of Sprague-Dawley rats with any Leydig cell tumor (unilateral, bilateral, or multifocal) following lifetime exposure to DEHP at doses of 300 mg/kg/day. In contrast, Ganning et al. (1991) did not observe any testicular tumors in male Sprague-Dawley rats following exposure to doses up 1,400 mg/kg/day for 102 weeks; however, only 7–18 animals were included in each dose group. Due to low animal number, it is unclear if the study design was adequate to assess potential carcinogenicity. Increased incidences of testicular tumors were not observed in other rat species following chronic exposure to doses up to 789 mg/kg/day (Carpenter et al. 1953; David et al. 1999, 2000a; Kluwe et al. 1982a, 1982b, 1985; NTP 1982), in male B6C3F1 mice at doses up to 1,325 mg/kg/day (David et al. 1999, 2000b; Kluwe et al. 1982a, 1982b, 1985; NTP 1982), in guinea pigs at doses up to 64 mg/kg/day (Carpenter et al. 1953), or in dogs at doses up to 56.6 mg/kg/day (Carpenter et al. 1953).

In a developmental study, the incidences of prostate cancer or precursor lesions were not increased in adult Sprague-Dawley rat offspring following exposure to doses up to 1 mg/kg/day from GD 7 to PND 21 (Wang et al. 2017a). However, the cancer analysis is limited due to small animal number (11/group),

which may have been inadequate to detect a significant effect for a lesion with high background incidence.

2.20 GENOTOXICITY

As discussed below and shown in Tables 2-18, 2-19, 2-20, and 2-21, DEHP has been extensively tested in a variety of genotoxicity assays. Evidence suggests that DEHP is not mutagenic to bacterial or mammalian cells; however, there is limited evidence that it may damage DNA and/or result in chromosomal abnormalities (either directly or indirectly via oxidative stress mechanisms), and it has been shown to induce morphological transformation. The weight of evidence from these assays indicates that DEHP is not a potent genotoxin but may lead to genotoxic effects secondary to oxidative stress.

Table 2-18. Genotoxicity of DEHP *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Prokaryotic organisms				
Salmonella typhimurium (TA98, TA100, TA1535, TA1538)	Gene mutation	–	–	Agarwal et al. 1985
S. typhimurium (NS)	Gene mutation	–	–	Astill et al. 1986
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	–	–	Kirby et al. 1983
S. typhimurium (TA100)	Gene mutation	–	+	Kozumbo et al. 1982
S. typhimurium (TA98)	Gene mutation	–	–	Sato et al. 1994
S. typhimurium (TA102)	Gene mutation	–	–	Schmezer et al. 1988
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	–	–	Simmon et al. 1977
S. typhimurium (TA100)	Gene mutation	–	–	Seed 1982
S. typhimurium (TA100)	Gene mutation	+	NS	Tomita et al. 1982b
S. typhimurium (TA98, TA100)	Gene mutation	–	–	Yoshikawa et al. 1983
S. typhimurium (TA98, TA1537)	Gene mutation	–	NS	Kanode et al. 2017
S. typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	Lee et al. 2019b
S. typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	Zeiger et al. 1985
Escherichia coli PQ37	Gene mutation	–	–	Sato et al. 1994

Table 2-18. Genotoxicity of DEHP *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
E. coli WP2UVRA$^+$	Gene mutation	–	–	Yoshikawa et al. 1983
E. coli WP2UVRA	Gene mutation	–	–	Yoshikawa et al. 1983
E. coli WP2UVRA	Gene mutation	-	-	Lee et al. 2019b
S. typhimurium (TA1535/psk 1002)	DNA damage	+	–	Okai and Higashi-Okai 2000
Bacillus subtilis (rec assay)	DNA damage	+	–	Tomita et al. 1982b
S. typhimurium (TA100)	Azaguanine resistance	–	–	Seed 1982
Eukaryotic organisms				
Saccharomyces cerevisae (XV185-14C, D7, RM52, D6, D5, D6-1)	Gene mutation	–	–	Parry et al. 1985
Saccharomyces cerevisiae (JD1, D7-144, D7)	Gene conversion	–	–	Parry et al. 1985
S. cerevisiae (D61M, D6)	Mitotic aneuploidy	+	+	Parry et al. 1985
S. cerevisiae (D61M, D6)	Mitotic segregation	–	–	Parry et al. 1985
Schizosaccharomyces pombe (P1)	Gene mutation	–	–	Parry et al. 1985
Aspergillus niger (P1)	Mitotic segregation	–	NS	Parry et al. 1985
Mammalian cells				
Mouse lymphoma cells	Mutagenicity	–	–	Astill et al. 1986
Mouse lymphoma cells	Mutagenicity	–	–	Kirby et al. 1983
Mouse lymphoma cells	Mutagenicity	±[a]	–	Oberly et al. 1985
Mouse lymphoma cells	Mutagenicity	–	–	Tennant et al. 1987
Human leukocytes	DNA damage	–	+	Anderson et al. 1999
Human lymphocytes	DNA damage	–	+	Anderson et al. 1999
Human HeLa cells	DNA damage	NS	+	Park and Choi 2007
Human HepG2 cells	DNA damage	NS	+	Choi et al. 2010
Human LNCaP prostate adenocarcinoma cells	DNA damage	NS	+	Erkekoglu et al. 2010a
Human HepaRG cells	DNA damage	–	NA	Le Hegarat et al. 2014
Human thyroid carcinoma	DNA damage	NS	+	Kim et al. 2019a
Mouse MA-10 Leydig tumor cells	DNA damage	NS	+	Erkekoglu et al. 2010b
Mouse lung cells	DNA damage	NS	+	Wang et al. 2014
Rat hepatocytes	DNA damage	–	NA	Schmezer et al. 1988
Hamster hepatocytes	DNA damage	–	NA	Schmezer et al. 1988
CHO cells	DNA damage	–	–	Douglas et al. 1986
Human hepatocytes	DNA repair	–	NA	Butterworth et al. 1984
Mouse hepatocytes	DNA repair	–	NA	Smith-Oliver and Butterworth 1987

Table 2-18. Genotoxicity of DEHP *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Rat hepatocytes	DNA repair	–	NA	Astill et al. 1986
Rat hepatocytes	DNA repair	–	NA	Butterworth 1984
Rat hepatocytes	DNA repair	–	NA	Hodgson et al. 1982
Rat hepatocytes	DNA repair	–	NA	Kornbrust et al. 1984
Rat hepatocytes	DNA repair	–	NA	Probst and Hill 1985
Chinese hamster V79 fibroblasts	DNA repair	–	NA	Kornbrust et al. 1984
Human HepaRG cells	Micronuclei	–	NA	Le Hegarat et al. 2014
Human TK6 lymphoblastoid cells	Micronuclei	NS	–	Sobol et al. 2012
Rat RL4 liver cells	Sister chromatid exchange	–	NA	Priston and Dean 1985
CHO cells	Sister chromatid exchange	NS	–	Abe and Sasaki 1977
CHO cells	Sister chromatid exchange	–	–	Douglas et al. 1986
CHO cells	Sister chromatid exchange	NS	–	Phillips et al. 1982
CHO cells	Sister chromatid exchange	NS	+	Tennant et al. 1987
Human hepatocytes	Chromosomal aberrations	–	NA	Turner et al. 1974
Human leucocytes	Chromosomal aberrations	–	NA	Stenchever et al. 1976
Rat RL4 liver cells	Chromosomal aberrations	–	NA	Priston and Dean 1985
CHO cells	Chromosomal aberrations	NS	–	Phillips et al. 1982
CHO cells	Chromosomal aberrations	NS	–	Tennant et al. 1987
Chinese hamster lung (CHL/OU)	Chromosomal aberrations	–	–	Lee et al. 2019b
SHE cells	Chromosomal aberrations	–	–	Tsutsui et al. 1993
CH SV40-transformed liver cells	Selective DNA amplification	–	NA	Schmezer et al. 1988
Mouse JB6 epidermal cells	Cell transformation	+	NA	Diwan et al. 1985
Mouse C3H/10T1/2 fibroblasts	Cell transformation	NS	–	Sanchez et al. 1987
Mouse BALB 3T3 cells	Cell transformation	–	–	Astill et al. 1986
SHE cells	Cell transformation	NS	+	LeBoeuf et al. 1996; Mauthe et al. 2001
SHE cells	Cell transformation	NS	+	Mikalsen et al. 1990
SHE cells	Cell transformation	NS	+	Pant et al. 2010
SHE cells	Cell transformation	NS	+	Sanner and Rivedal 1985
SHE cells	Cell transformation	+	±	Tsutsui et al. 1993
Rat hepatocytes	DNA binding	–	NA	Gupta et al. 1985

Table 2-18. Genotoxicity of DEHP *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Human fetal pulmonary cells	Aneuploidy	–	NA	Stenchever et al. 1976
Rat RL4 liver cells	Polyploidy	–	NA	Priston and Dean 1985

[a]Mutagenic effect coincident with cytotoxicity.

– = negative result; + = positive result; ± = equivocal result; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; NA = not applicable to mammalian cell cultures with endogenous metabolic activity; NS = not specified; SHE = Syrian hamster embryo

Table 2-19. Genotoxicity of MEHP *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Prokaryotic organisms				
Salmonella typhimurium (TA98, TA100, TA1535, TA1538)	Gene mutation	−	−	Agarwal et al. 1985
S. typhimurium (NS)	Gene mutation	−	−	Astill et al. 1986
S. typhimurium (TA97, TA98, TA100, TA102)	Gene mutation	−	−	Dirven et al. 1991
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	−	−	Kirby et al. 1983
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	−	−	Ruddick et al. 1981
S. typhimurium (TA100, TA102)	Gene mutation	−	−	Schmezer et al. 1988
S. typhimurium (TA100)	Gene mutation	−	±	Tomita et al. 1982b
S. typhimurium (TA98, TA100)	Gene mutation	−	−	Yoshikawa et al. 1983
S. typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	−	−	Zeiger et al. 1985
Escherichia coli (WP2 B/r)	Gene mutation	NS	±[a]	Tomita et al. 1982b
E. coli (WP2 *try*$^-$ [*UvrA*$^+$ *and UvrA*$^-$])	Gene mutation	−	−	Yoshikawa et al. 1983
Bacillus subtilis (H17, M45)	DNA damage (Rec assay)	NS	+	Tomita et al. 1982b
Mammalian cells				
Mouse lymphoma cells L5178Y (tk$^+$/tk$^-$)	Mutagenicity	−	−	Kirby et al. 1983
CHO cells	Mutagenicity	NS	−	Phillips et al. 1982

Table 2-19. Genotoxicity of MEHP *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
CHO cells (AS52)	Mutagenicity	NS	+	Chang et al. 2017c
Human leukocytes	DNA damage	NS	+	Anderson et al. 1999
Human LNCaP prostatic cancer cells	DNA damage	NS	+	Erkekoglu et al. 2010a
Mouse MA-10 Leydig tumor cells	DNA damage	NS	+	Erkekoglu et al. 2010b
Human peripheral lymphocytes	DNA damage	NS	+	Kleinsasser et al. 2004
Human nasal mucosa cells	DNA damage	NS	+	Kleinsasser et al. 2004
CHO cells (AS52)	DNA damage	NS	+	Chang et al. 2017c
Human HepG2 cells	Oxidative DNA damage	NS	+	Yang et al. 2012
Human primary hepatocytes	DNA repair	−	NA	Butterworth et al. 1984
Rat primary hepatocytes	DNA repair	−	NA	Cattley et al. 1986
Mouse primary hepatocytes	DNA repair	−	NA	Smith-Oliver and Butterworth 1987
Hamster SV40 transformed cells	DNA amplification	NS	−	Schmezer et al. 1988
Chinese hamster V79 fibroblasts	Sister chromatid exchange	NS	+	Tomita et al. 1982b
Rat RL4 liver cells	Chromosomal aberrations	NS	+	Phillips et al. 1986
CHO cells	Chromosomal aberrations	+	+	Phillips et al. 1986
CHO cells	Chromosomal aberrations	NS	+	Phillips et al. 1982
SHE cells	Chromosomal aberrations	+	−	Tsutsui et al. 1993
CHO transformed cells	Gene mutation	NS	+	Chang et al. 2017c
Mouse BALB 3T3 cells	Cell transformation	–	–	Astill et al. 1986
Mouse C3H/10T1/2 fibroblasts	Cell transformation	NS	–	Sanchez et al. 1987
SHE cells	Cell transformation	NS	+	Mikalsen et al. 1990
SHE cells	Cell transformation	+	−	Tsutsui et al. 1993

[a]Mutagenic effect coincident with cytotoxicity.

– = negative result; + = positive result; ± = equivocal result; DNA = deoxyribonucleic acid; NA = not applicable to mammalian cell cultures with endogenous metabolic activity; NS = not specified

Table 2-20. Genotoxicity of DEHP *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Mammals			
Mouse (subcutaneous)	Dominant lethal test	+	Autian 1982
Mouse (gavage)	Dominant lethal test	–	Rushbrook et al. 1982
Mouse (intraperitoneal)	Dominant lethal test	+	Singh et al. 1974
Rat (*gpt* delta transgenic) (diet)	Gene mutation in liver	–	Kanki et al. 2005
Mouse (lacZ transgenic) (NS)	Gene mutation in liver	+	Boerrigter 2004
Mouse (lacZ transgenic) (NS)	Gene mutation in kidney or spleen	–	Boerrigter 2004
Hamster embryo (gavage; via placenta)	8AG/6TG-resistant mutation	+	Tomita et al. 1982b
Mouse (NS)	Micronuclei in bone marrow	–	Astill et al. 1986
Mouse (intraperitoneal)	Micronuclei in bone marrow	–	Douglas et al. 1986
Mouse (Oral)	Micronuclei in bone marrow	–	Lee et al. 2019b
Human (unknown)	DNA damage in sperm and granulosa cells	+	Al-Saleh et al. 2019b
Human (unknown)	DNA damage in peripheral blood cells	–	Franken et al. 2017
Rat (gavage, diet)	DNA damage in liver	–	Butterworth et al. 1984
Rat (diet)	DNA damage in liver	–	Tamura et al. 1991
Rat (diet)	DNA damage in liver	−	Pogribny et al. 2008
Rat (gavage)	DNA damage in sperm	+	Hsu et al. 2016
Rat (gavage)	DNA damage in blood lymphocytes and sperm	+	Karabulut and Barlas 2018
Rat (gavage)	DNA damage in thyroid	+	Kim et al. 2019a
Mouse (pipette)	Oxidative DNA damage in brain	+	Barakat et al. 2018
Mouse (gavage)	Oxidative DNA damage in oocytes	+	Lu et al. 2019
Rat (diet)	DNA base modification in liver	–	Cattley and Glover 1993
Rat (diet)	DNA base modification in liver	+	Takagi et al. 1990
Rat (gavage, diet)	DNA repair in liver	–	Butterworth et al. 1984
Rat (diet)	DNA repair in liver	–	Cattley et al. 1988
Rat (gavage, diet)	DNA repair in liver	–	Kornbrust et al. 1984
Rat (gavage)	DNA repair in liver	+	Hayashi et al. 1998
Mouse (gavage, diet)	DNA repair in liver	–	Smith-Oliver and Butterworth 1987
Rat (diet)	DNA binding in liver	+	Albro et al. 1982a
Rat (gavage)	DNA binding in liver	–	Gupta et al. 1985

Table 2-20. Genotoxicity of DEHP *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Rat (gavage, diet)	DNA binding in liver	–	Lutz 1986; Von Däniken et al. 1984
Human (occupational)	Chromosomal aberrations in leucocytes	–	Thiess and Fleig 1978
Rat (gavage)	Chromosomal aberrations in bone marrow	–	Putman et al. 1983
Hamster embryo (gavage; via placenta)	Chromosomal aberrations	+	Tomita et al. 1982b
Hamster embryo (gavage; via placenta)	Cell transformation	+	Tomita et al. 1982b
Rat embryo (intraperitoneal; via placenta)	Mitotic recombination	+	Fahrig and Steinkamp-Zucht 1996
Rat (diet)	Tetraploid nuclei in liver	+	Ahmed et al. 1989
Host-meditated assay			
Salmonella typhimurium (TA100); (rat host-meditated)	Gene mutation	–	Kozumbo et al. 1982
Eukaryotic organisms			
Drosophila melanogaster (feeding)	Mitotic recombination	–	Vogel and Nivard 1993
D. melanogaster (injection)	Sex linked recessive lethal	–	Yoon et al. 1985

– = negative result; + = positive result; DNA = deoxyribonucleic acid; *gpt* = guanine phosphoribosyltransferase; NS = not specified

Table 2-21. Genotoxicity of MEHP *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Mammals			
Rat (gavage)	DNA damage in liver	−	Elliott and Elcombe 1987
Rat (gavage)	Chromosomal aberrations in bone marrow	−	Putman et al. 1983
Hamster embryo (gavage; via placenta)	Chromosomal aberrations	+	Tomita et al. 1982b
Hamster embryo (gavage; via placenta)	Cell transformation	+	Tomita et al. 1982b
Hamster embryo (gavage; via placenta	8AG/6TG-resistant mutation	+	Tomita et al. 1982b

– = negative result; + = positive result

As shown in Tables 2-18 and 2-19, 30 *in vitro* assays indicate that neither DEHP nor its metabolite, MEHP, is mutagenic to bacteria, eukaryotic organisms, or mammalian cells, either with or without

metabolic activation. The few isolated positive results have not been replicated, were borderline responses, and/or were accompanied by cytotoxicity (Chang et al. 2017c; Kim et al. 2019a; Kozumbo et al. 1982; Oberly et al. 1985; Tomita et al. 1982b). In a host-mediated assay, urine from rats injected with DEHP was not mutagenic to bacterial cells (Kozumbo et al. 1982). Additionally, DEHP did not induce sexed-linked recessive lethal mutations in *Drosophila melanogaster* (Yoon et al. 1985). MEHP produced a mutagenic response in Chinese hamster ovary (CHO) cells carrying a single functional copy of the bacterial *gpt* gene (AS52 cells). This effect was reversed by addition of N-acetyl cysteine, suggesting that the mutagenic effect resulted from oxidative stress (Chang et al. 2017c).

In vivo mammalian assays are limited and reported mixed results. 8AG/6TG-resistant mutations were observed in embryonic DNA collected from hamster dams exposed to a single gavage dose up to 15,000 mg/kg DEHP or MEHP during gestation (Tomita et al. 1982b). In transgenic animal lines, exposure to DEHP resulted in gene mutations in the liver of *lacZ* transgenic mice, but not in the kidney or spleen (Boerrigter 2004), and not in the liver of guanine phosphoribosyltransferase (*gpt)* delta transgenic rats (Kanki et al. 2005). Exposing Wistar or Sprague-Dawley rats orally to DEHP resulted in DNA damage to blood lymphocytes, sperm cells, and thyroid tissue (Hsu et al. 2016; Karabulut and Barlas 2018; Kim et al. 2019a). DNA damage in granulosa and sperm cells and markers of oxidative DNA damage in seminal plasma and follicular fluid were associated with DEHP urinary metabolites in a prospective birth cohort seminal plasma (Al-Saleh et al. 2019b). In adolescents, a positive association was observed between markers of oxidative DNA damage and DEHP urinary metabolites; however, DNA damage in peripheral blood cells were not associated with DEHP exposure (Franken et al. 2017). Oxidative DNA damage was also observed in mouse oocytes following oral exposure to DEHP (Lu et al. 2019) and in the brain of adult mouse offspring following maternal oral exposure to DEHP during gestation (Barakat et al. 2018). Dominant lethal mutations were increased in mice that were exposed to DEHP by injection at dose levels that also resulted in decreased fertility, but not when exposure was by oral administration (Autian 1982; Rushbrook et al. 1982; Singh et al. 1974). The results of these studies are not necessarily indicative of genotoxicity because DEHP has not been shown to induce DNA lesions in most studies, and positive findings can be interpreted in different ways. For example, dominant lethal tests can be interpreted as indicating that the test chemical altered gene expression (i.e., by epigenetically shutting off the marker gene) rather than by mutation.

Spot tests were conducted in which mouse embryos heterozygous for recessive coat color mutations were exposed *in utero* to the direct monofunctional alkylating mutagen ethylnitrosourea (ENU), either alone or followed by intraperitoneal injection of the pregnant dam with DEHP (Fahrig and Steinkamp-Zucht

1996). DEHP, in combination with ENU, resulted in an increase in the number of spots indicative of reciprocal recombination, compared to ENU treatment alone. Conversely, DEHP alone resulted in a reduction in the number of spots that arose from ENU-induced gene mutations. These findings are not necessarily indicative of interference with DNA repair processes because DEHP could have induced altered spots epigenetically rather than by mutagenic means. As discussed by Trosko (1997, 2001), mutation assays are often misinterpreted to give false positives results for epigenetic (nonmutagenic) agents.

Binding of DEHP to DNA in rat liver was reported by Albro et al. (1982a, 1982b) following *in vivo* exposure, but was not observed by other investigators (Gupta et al. 1985; Lutz 1986; Von Däniken et al. 1984). *In vitro,* DEHP did not bind to DNA in rat hepatocytes (Gupta et al. 1985). However, several studies reported DNA damage (strand breakage) in cultured human, mouse, or bacterial cells exposed to DEHP or MEHP without metabolic activation (Anderson et al. 1999; Chang et al. 2017c; Choi et al. 2010; Erkekoglu et al. 2010a, 2010b; Kleinsasser et al. 2004; Okai and Higashi-Okai 2000; Park and Choi 2007; Tomita et al. 1982b; Wang et al. 2014). Yang et al. (2012) specifically reported oxidative DNA damage in human HepG2 cells exposed to MEHP without metabolic activation. DNA damage was reversed by the addition of N-acetyl cysteine, suggesting a role for oxidative stress in this process (Chang et al. 2017c). As shown in Tables 2-18 and 2-19, 14 studies reported that DEHP and MEHP did not cause DNA damage or repair in human, rat, mouse, or hamster cells with metabolic capacity or cultured cells with exogenous metabolic activation. Hayashi et al. (1998) reported evidence of DNA repair (increased expression of the post-translational modifying enzyme poly[ADP-ribose] polymerase) in the livers of rats exposed to 2,000 mg/kg/day DEHP via gavage for 7 days or 1,800 mg/kg/day DEHP in feed for up to 97 weeks. However, eight *in vivo* studies did not observe DNA damage or repair in rat livers following exposure to DEHP or MEHP (Tables 2-20 and 2-21). 8-Hydroxydeoxyguanosine was detected in hepatic DNA in rats exposed to 1,200 mg/kg/day DEHP for 2 weeks, indicating a potential for DNA damage secondary to oxidative stress (Takagi et al. 1990); however, Cattley and Glover (1993) did not confirm this finding in similarly treated rats exposed for up to 22 weeks.

Chromosomal aberrations were observed in embryonic DNA collected from hamster dams exposed to a single gavage dose up to 15,000 mg/kg DEHP or MEHP during gestation (Tomita et al. 1982b). However, increased frequencies of chromosomal aberrations were not observed in peripheral leukocytes collected from 10 workers occupationally exposed to DEHP at air concentrations of 0.0006–0.01 ppm for 10–30 years, compared with 20 control workers (Thiess and Fleig 1978). Additionally, chromosomal aberrations were not induced in rat bone marrow following oral exposure to DEHP or MEHP (Putman et

al. 1983). Six *in vitro* mammalian studies reported a lack of chromosomal aberrations following exposure to DEHP (Table 2-18). However, findings following *in vitro* MEHP exposure were mixed, with evidence of chromosomal aberrations in 1/1 rat RL4 liver cell assay (without activation), 2/4 CHO cells assays (with and without metabolic activation), and 1/1 SHE cell assays (with activation) (Phillips et al. 1982, 1986; Tsutsui et al. 1993).

No clear evidence of micronucleus induction was observed following exposure to DEHP or MEHP in mouse bone marrow assays *in vivo* (Astill et al. 1986; Douglas et al. 1986) or in human cells exposed *in vitro* (Le Hegarat et al. 2014; Sobol et al. 2012). Similarly, the majority of *in vitro* studies did not observe increases in sister chromatid exchanges in mammalian cells exposed to DEHP, with or without metabolic activation (Abe and Sasaki 1977; Douglas et al. 1986; Phillips et al. 1982; Priston and Dean 1985), although a few studies reported equivocal or positive results in mammalian cells exposed to DEHP or MEHP without metabolic activation (Tennant et al. 1987; Tomita et al. 1982b).

Cell transformation was observed in embryonic DNA collected from hamster dams exposed to a single gavage dose up to 15,000 mg/kg DEHP or MEHP during gestation (Tomita et al. 1982b). Cell transformation was observed in all seven *in vitro* Syrian hamster embryo (SHE) cell assays with DEHP or MEHP, both with and without metabolic activation (Tables 2-18 and 2-19). Cell transformation was not observed in *in vitro* assays with mouse fibroblasts or 3T3 cell lines exposed to DEHP or MEHP (Astill et al. 1986; Sanchez et al. 1987); however, DEHP induced cell transformation in mouse epidermal cells exposed to DEHP with (but not without) metabolic activation (Diwan et al. 1985).

Rats that were exposed to 1,000 mg/kg/day DEHP for periods of 3 or 7 days alternating with 7-day withdrawal periods had increased liver cell division and numbers of tetraploid nuclei during the exposure periods (Ahmed et al. 1989). During the withdrawal periods in the latter study, the cell number declined, and degenerated cells appeared to be those containing the tetraploid nuclei. Cells are more vulnerable to irreversible mutagenic alterations during a period of rapid cell division (Marx 1990), and it has been postulated that the carcinogenicity of DEHP might be a consequence of its induction of cell division in the liver in the presence of other mutagens (Smith-Oliver and Butterworth 1987). The available evidence supports the interpretation that DEHP is mitogenic, not mutagenic, because mutagens, by inducing DNA lesions, would inhibit DNA synthesis and cell proliferation. In general, evidence for DNA amplification and aneu/polyploidy has not been observed in mammalian cells exposed to DEHP or MEHP *in vitro* (Priston and Dean 1985; Schmezer et al. 1988; Stenchever et al. 1976); however, mitotic aneuploidy was observed in *Saccharomyces cerevisiae* following exposure to DEHP both with and without metabolic

activation (Parry et al. 1985). Gene conversion and/or mitotic segregation were not observed in *S. cerevisiae* or *Aspergillus niger* (Parry et al. 1985). Additionally, mitotic recombination was not observed in *D. melanogaster* fed DEHP (Vogel and Nivard 1993).