

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, animal oral studies are presented in Table 2-2 and Figure 2-3, and animal dermal studies are presented in Table 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 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

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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

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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 PPAR α (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

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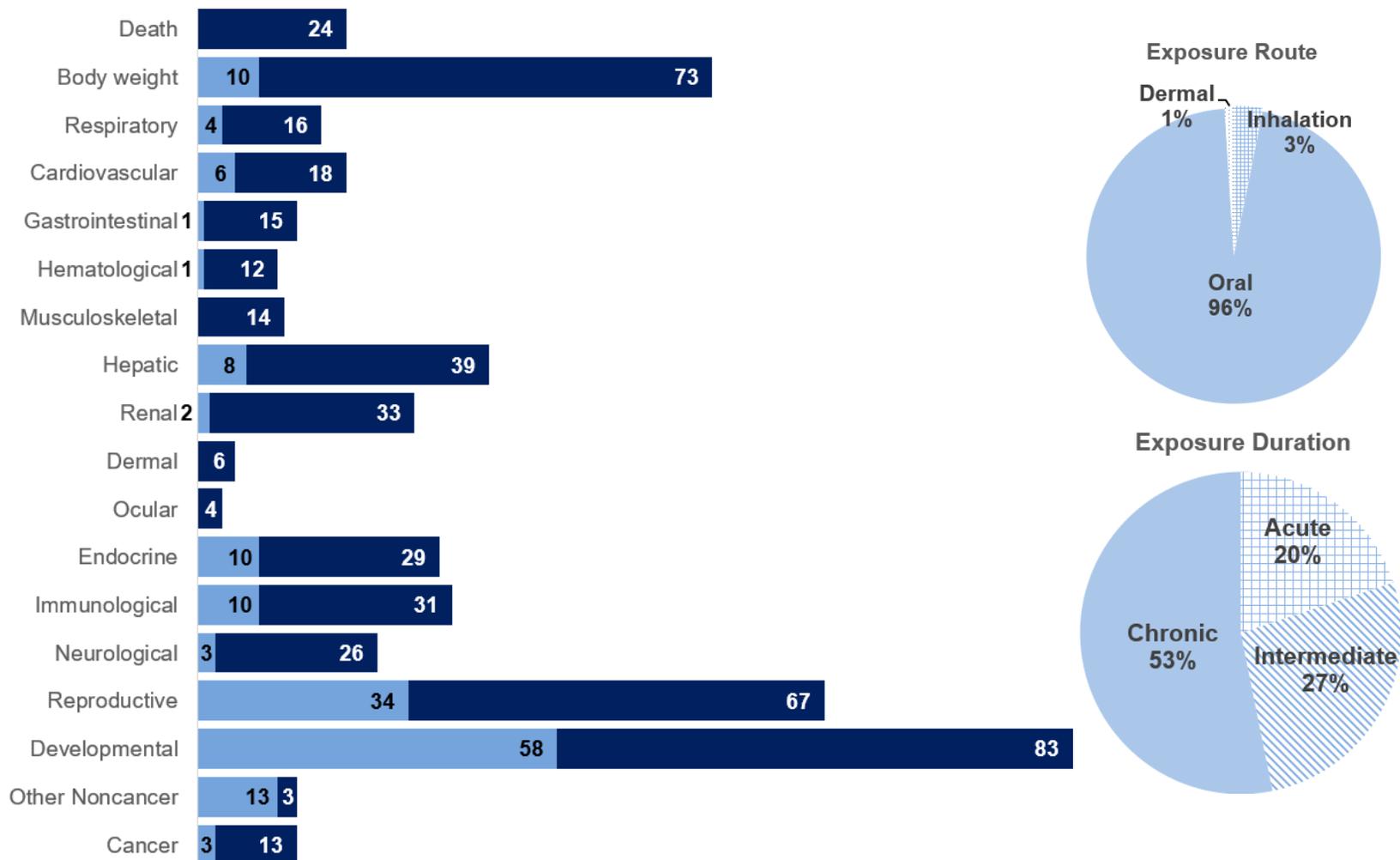
is a sensitive target of DEHP toxicity following oral exposure. However, most of the available studies observed kidney damage only at high doses.

- **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.
- **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 are limited to a single study in 439 couples and 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 descent 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 development of the reproductive system following early life exposure are particularly sensitive targets of DEHP toxicity.

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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 285 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints.

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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, FX, MX, TG	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		Decreased tidal volume, increased respiratory rate
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 Resp Cardio Hemato Musc/skel Hepatic Renal Endocr Immuno Neuro Repro	63 3 63 63 63 63 63 63 63 63 63	63		Transient increases in lung weight, foam cell proliferation, and thickening of alveolar septa Increased relative liver weight at 63 ppm ^b
Klimisch et al. 1991, 1992									

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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		Increased 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		Increased plasma testosterone, increased relative seminal vesicle weight
Kurahashi et al. 2005									
6	Rat (Wistar) 10 F	21 days (PNDs 22–42) 6 hours/day 5 days/week (WB)	0, 0.3, 1.6	DX	Develop		0.3 ^c		Accelerated vaginal opening and first estrus at ≥0.3 ppm; increased serum estradiol and 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		Accelerated vaginal opening and first estrus at ≥0.3 ppm; irregular estrous cycles and ~10% decrease in terminal body weight at 1.6 ppm
Ma et al. 2006									

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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, OF, OW	Bd wt Hepatic Immuno	0.81 0.81 0.11	0.81		Enhanced immune response to OVA challenge in sensitized animals

Larsen et al. 2007 [OVA-sensitized mice]

^aThe number corresponds to entries in Figure 2-2.

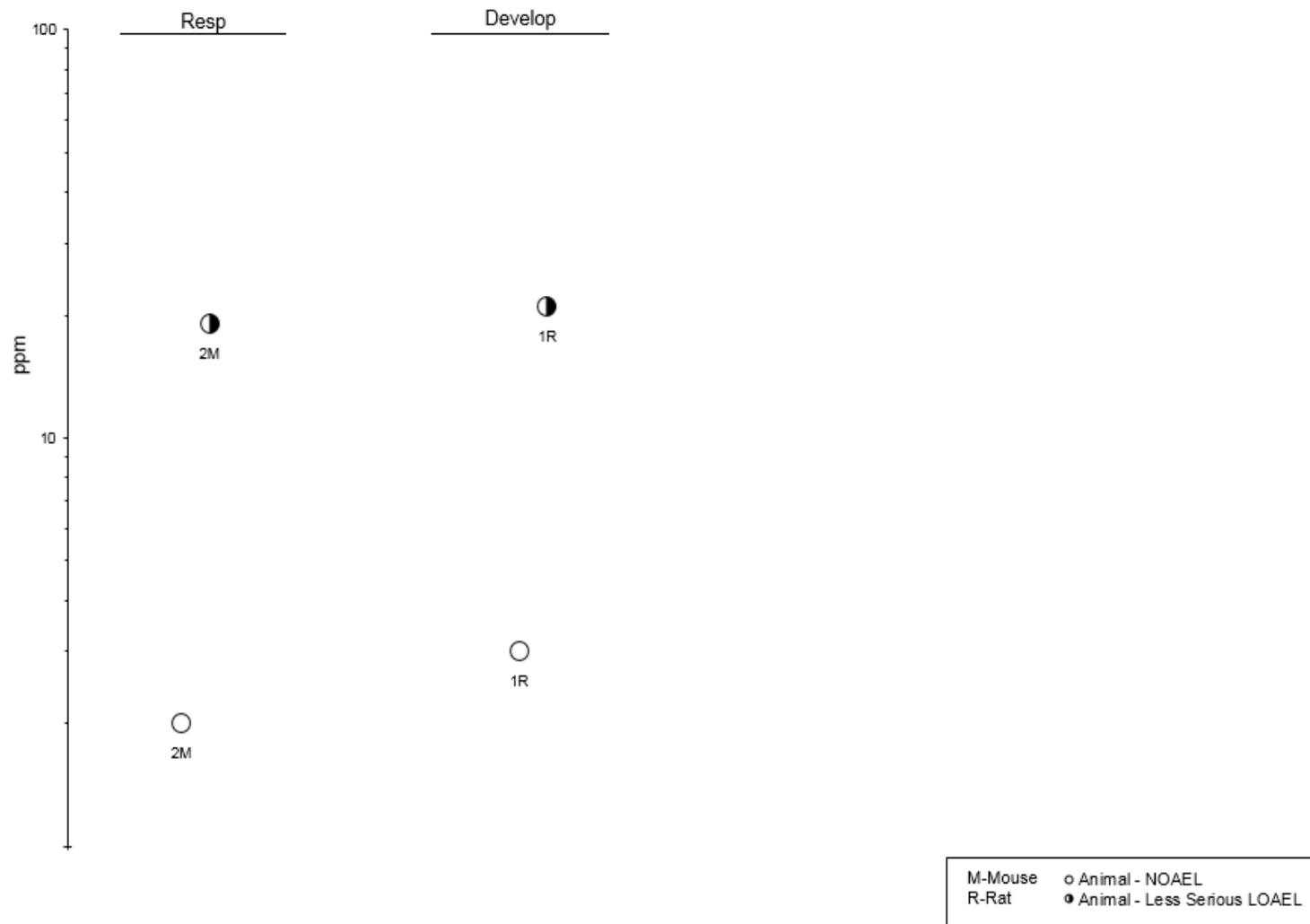
^bHepatic effects associated with hepatomegaly (elevated liver weight, hypertrophy, enzyme induction, and/or peroxisome proliferation) are not considered adverse unless hepatocellular degenerative or necrotic changes or evidence of biliary or other liver cell damage were also present (Hall et al. 2012). The lowest doses associated with hepatomegaly endpoints are noted in the LSE tables even though the dose levels are considered NOAELs.

^cUsed to derive a provisional 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 × 6 hours/24 hours × 5 days/7days × 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 a provisional 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); FX = fetal toxicity; GD = gestational day; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect level; LSE = levels of significant exposure; M = male(s); Musc/skel = musculoskeletal; MX = maternal toxicity; (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; TG = teratogenicity; (WB) = whole-body

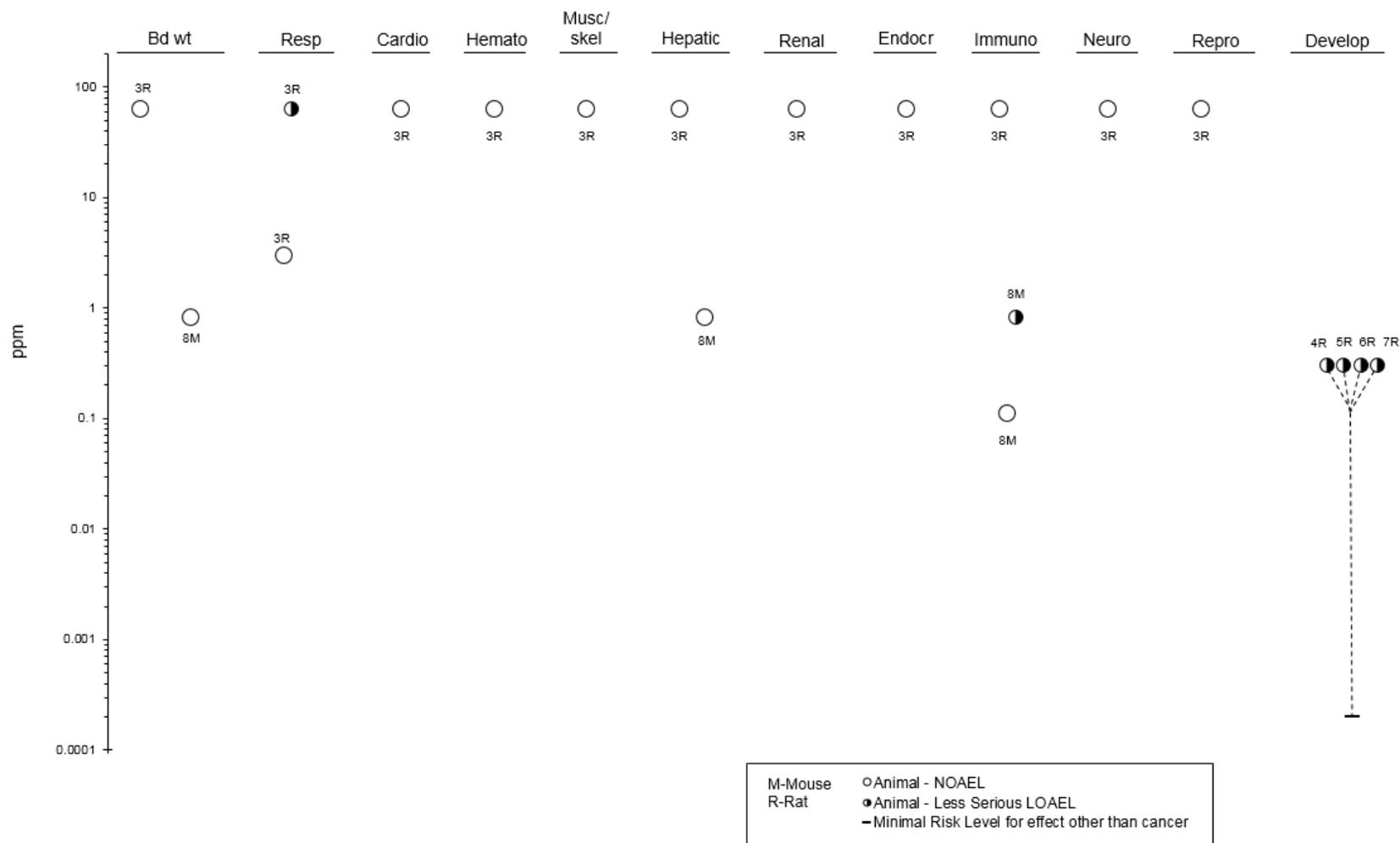
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Figure 2-2. Levels of Significant Exposure to DEHP – Inhalation
Acute (≤ 14 days)



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



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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	Human 1 M	Once (C)	71.4, 142.9	CS	Gastro	71.4	142.9		Gastrointestinal distress
Shaffer et al. 1945									
2	Monkey (Cynomolgus) 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	Monkey (Marmoset) 5 M, 5 F	14 days (GO)	0, 2,000	BC, BI, BW, HE, HP, OW	Hemato Hepatic Renal Neuro Repro	2,000 2,000 2,000 2,000 2,000			
ICI Americas Inc. 1982; Rhodes et al. 1986									
4	Rat (Long-Evans) 10 M	14 days PNDs 21–34 (GO)	0, 1, 10, 100, 200	DX	Develop	10	100		Reduced testosterone production in Leydig cells
Akingbemi et al. 2001									
5	Rat (Long-Evans) 10 M	14 days PNDs 35–48 (GO)	0, 1, 10, 100, 200	DX	Develop	1	10		Reduced testosterone production in Leydig cells; reduction in androgen biosynthesis enzymes
Akingbemi et al. 2001									

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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	
6	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				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
					Hepatic	85	530			
					Musc/skel	1,100				
					Renal	1,100				
					Endocr	1,100				
					Immuno	1,100				
					Neuro	1,100				
					Repro	1,100				
Astill et al. 1986										
7	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 $\geq 1,500$ mg/kg/day; increased liver weight and hepatocellular hypertrophy at all doses ^b	
					Endocr	5,000				
					Immuno	5,000				
Berman et al. 1995										
8	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 $\geq 1,500$ 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										
9	Rat (Sprague-Dawley) 8–10 F	10 days GD 12–PND 0 (GO)	0, 10, 100, 750	BW, DX	Bd wt	100		750	Maternal weight loss during exposure period ~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	
					Develop	10	100	750		
Chen et al. 2010										

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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
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									
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	1,000		Increased relative kidney weight; slight decrease absolute kidney weight
Dostal et al. 1987									
12	Rat (Sprague-Dawley) 6–10 M	5 days PNDs 86–90 (GO)		BI, OW	Renal	100	1,000		Increased relative kidney weight; slight decrease absolute kidney weight
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									

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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 (Long-Evans) 19–38 M	14 d 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									
19	Rat (Long-Evans) 6 M	7 days (GO)	0, 10, 750	BW, HP	Bd wt Repro	750	10		Increased Leydig cell number in testes
Guo et al. 2013									
20	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									
21	Rat (Sprague-Dawley) 3–6 F	5 days GDs 14–18 (GO)	0, 100, 300, 500, 625, 750, 875	BW, DX	Bd wt Develop	500 100	300	625	>50% decrease in maternal body weight gain Decreased fetal testicular testosterone production
Hannas et al. 2011									
22	Rat (Wistar) 3–6 F	5 days GDs 14–18 (GO)	0, 100, 300, 500, 625, 750, 875	BW, DX	Bd wt Develop	500 100	300	625	>30% decrease in maternal body weight gain Decreased fetal testicular testosterone production
Hannas et al. 2011									
23	Rat (Sprague-Dawley) 6–8 F	5 days GDs 14–18 (GO)	0, 100, 300, 600, 900	DX	Develop		100		Decreased fetal testicular testosterone production
Furr et al. 2014; Hannas et al. 2011									

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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
24	Rat (Wistar) 9–10 F	9 days GDs 6–15 (GO)	0, 40, 200, 1,000	BW, CS, DX, OW, TG	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		Decreased fetal testicular testosterone production
Howdeshell et al. 2008									
26	Rat (Sprague-Dawley) 8 F	7 days GDs 13–19 (GO)	0, 10, 100	DX	Develop		10		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									
27	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									
28	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]									

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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
29	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									
30	Rat (Long-Evans) 8 M	14 days (GO)	0, 10, 750	BC, OF	Bd wt Repro	750	10		Increased Leydig cell number/proliferation following EDS elimination of Leydig cells
Li et al. 2012a									
31	Rat (Sprague-Dawley) 3 F	8 days GD 14–PND 0 (GO)	0, 20, 50, 100, 300, 750	DX	Develop	50 M 100 F	100 M 300 F		Decreased serum testosterone and aldosterone at ≥100 mg/kg/day; reduced adrenal weight at 750 mg/kg/day 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 measured in adult (PND 60) offspring]									
32	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									
33	Rat (Fischer-344) 8 F	Once (GO)	0, 150, 500, 1,500, 5,000	BH, CS	Neuro	1,500	5,000		Signs of general debilitation
Moser et al. 1995									
34	Rat (Fischer-344) 8 F	14 days (GO)	0, 50, 150, 500, 1,500	BH, CS	Neuro	1,500			
Moser et al. 1995									

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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
35	Rat (Fischer-344) 10 F	10 days (GO)	0, 50, 100, 150, 200	BH, BW, CS	Bd wt Neuro	200 200			
Moser et al. 2003									
36	Rat (Fischer-344) 5 M, 5 F	14 days PNDs 38.5–52.5 (F)	M: 0, 670, 1,300, 2,700, 5,700, 12,000 F: 0, 730, 1,500, 3,000, 6,200, 12,000	DX, LE	Death Develop			12,000	2/5 males and 4/5 females died 22–53% decrease in body weight at ≥5,700 mg/kg/day; food consumption not measured
NTP 1982									
37	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; 12–21% decreased body weight and increased adipose tissue at ≥10 mg/kg/day in adult offspring
Rajesh and Balasubramanian 2014a									
38	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									
39	Rat (Sprague-Dawley) 8–12 F	8 days GDs 12–19 (GO)	0, 50, 625	DX	Develop		50		Decreased fetal testosterone production
Saillenfait et al. 2013									
40	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									
41	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]									

2. HEALTH EFFECTS

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 (Sprague-Dawley) 8 F	11 days GDs 11–21 (GO)	0, 10, 100, 500	DX	Develop		10	500	Effects at PNDs 13–63: Sperm effects at ≥ 10 mg/kg/day; 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									
43	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]									
44	Rat (Sprague-Dawley) 10 F	4 days (GO)	0, 20, 200, 2,000	BW, OW	Bd wt Repro	2,000 2,000			
Zacharewski et al. 1998 [mature ovariectomized rats]									
45	Mouse (CD-1) 9–20 F	10 days GDs 9–18	0, 0.0005, 0.001, 0.005, 0.5, 50, 500 (micropipette)	BC, DX, MX	Repro Develop	500 0.5	50		Decreased fetal testes weight
Do et al. 2012									
46	Mouse (A/J) 10 M	2 weeks (F)	0, 12.3, 125	BW, FI, HP, OF, WI	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									
47	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									

2. HEALTH EFFECTS

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
48	Mouse (CD-1) 10 F	9 days GDs 11–19 (GO)	0, 25, 100	BW, DX, FI, HP, OW	Bd wt Hepatic Repro Develop	100 100 100	25		Transient liver lesions in PND 21 offspring (pyknotic nuclei, hepatocyte vacuolization)
Maranghi et al. 2010									
49	Mouse (C57BL/6N) 5 M	7 days (F)	0, 385, 1,250, 3,850	BI, BW, OW	Bd wt				17% decrease in final body weight at 3,850 mg/kg/day; food consumption not measured
Muhlenkamp and Gill 1998									
50	Mouse (B6C3F1) 5 M, 5 F	14 days PNDs 44–58 (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 Develop		11,000 F 20,000 M		4/5 died at 11,000 mg/kg/day, 5/5 died at 20,000 mg/kg/day 5/5 died 17–29% decrease in male body weight at ≥4,900 mg/kg/day and female body weight at ≥11,000 mg/kg/day; food consumption not measured
NTP 1982									
51	Mouse (C57BL/6) 6 M	10 days (F)	0, 180, 360	BW, OW, OF	Bd wt Immuno	360 360			
Sasaki et al. 2003									
52	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									
53	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									

2. HEALTH EFFECTS

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
54	Rabbit (NS) 4-5 M	7 days (GO)	0, 2,000	LE	Death			2,000	50% died
Parmar et al. 1988									
55	Rabbit (NS) NS	Once (G)	NS	CS, BW	Death			33,900	LD ₅₀
Shaffer et al. 1945									
INTERMEDIATE EXPOSURE									
56	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 Resp Cardio Gastro Hemato Musc/skel Hepatic Renal Dermal Ocular Endocr Repro	2,500 2,500 2,500 2,500 2,500 2,500 2,500 2,500 2,500 2,500 2,500 2,500			
Kurata et al. 1998									
57	Monkey (Cynomolgus) 3 M, 3-4 F	28 days (GO)	0, 1,000	BC, EA, HE, HP, OW	Hemato Hepatic Renal	1,000 1,000 1,000			
Satake et al. 2010									

2. HEALTH EFFECTS

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
58	Rat (Fischer-344) 24 M	60 days (F)	0, 17.5, 69.2, 284.1, 1,156.4	BC, BW, FI, HP, OF, 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									
59	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									
60	Rat (Long-Evans) 10 M	28 days PNDs 62–89 (GO)	0, 1, 10, 100, 200	BC, BW, HP, OF	Bd wt Repro	200 200			
Akingbemi et al. 2001									
61	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									
62	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									
63	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									

2. HEALTH EFFECTS

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
64	Rat (Long-Evans) 12 F	42 days GD 1 – PND 21 (W)	0, 3, 30	BM, BW, DX, MX, OF	Bd wt Repro Develop	30 30		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									
65	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 Musc/skel Hepatic Renal Endocr Immuno Neuro Repro	950 950	75	930	Decreased serum lipids, increased liver weight, enzyme induction at ≥75 mg/kg/day; hepatocellular hypertrophy and peroxisomal proliferation at 470 mg/kg/day Increased absolute and relative kidney weight
Astill et al. 1986									
66	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 Hepatic Renal Repro	1,224 11	105	2,101 1,224 M 2,101 M	38–44% decrease in body weight and 48–60% decreased in food consumption at ≥1,892 mg/kg/day 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 Decreased testicular weight and testicular atrophy
Barber et al. 1987; CMA 1984 [female reproductive organs not assessed]									

2. HEALTH EFFECTS

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
67	Rat (Sprague-Dawley) 17 M, 17 F	24 weeks (3-generation) 6 weeks pre mating through 3 weeks post-weaning of 3rd litter (F)	0.1, 0.58, 1.7, 5.9, 17, 57, 447, 659	BW, DX, FI, HP, OF, OW	Bd wt	57 M	447 M		10–19% decreased F1/F2 body weight; no change in food consumption
						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 ≥ 464.44 mg/kg/day and F1 pups at 658.82 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]									
68	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									
69	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									

2. HEALTH EFFECTS

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
70	Rat (Wistar) 3 F	36 days GD 1– PND 15 (W)	0, 3, 30	DX, MX	Repro Develop	30 3		30	Decreased testes weight and increased serum LH and FSH at PND 15
Carbone et al. 2012									
71	Rat (Wistar) 5 F	30 days PNDs 1–21 (via dam) PNDs 22–30 (W)	0, 30	DX	Develop	30			
Carbone et al. 2013									
72	Rat (Wistar) 5 F	45 days PNDs 1–21 (via dam) PND 22–45 (W)	0, 30	DX	Develop	30F		30 M	Increased anxiety-like behavior in elevated plus maze
Carbone et al. 2013									
73	Rat (Wistar) 5 F	60 days PNDs 1–21 (via dam) PNDs 22–60 (W)	0, 30	DX	Develop			30 M	Increased anxiety-like behavior in elevated plus maze, decreased serum testosterone, and increased serum LH
Carbone et al. 2013									
74	Rat (Sprague-Dawley) 8–10 F	31 days GD 12– PND 21 (GO)	0, 10, 100, 750	BW, DX	Bd wt Develop	750		10	>10% decrease in body weight at PND 21 at ≥10 mg/kg/day; >10% decrease in birth weight, increased thickness of alveolar septa, and increased interstitial lung tissue proportion at ≥100 mg/kg/day
Chen et al. 2010									
75	Rat (Wistar) 8–16 F	31 days GD 7– PND 16 (GO)	0, 3, 10, 30, 100	BI, BW, DX, MX, OF	Bd wt Repro Develop	100 100		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									

2. HEALTH EFFECTS

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) 8–16 F	31 days GD 7–PND 16 (GO)	0, 10, 30, 100, 300, 600, 900	BI, BW, DX, MX, OF	Bd wt Repro Develop	900 900	10		Decreased AGD, increased nipple retention, decreased adrenal gland and LABC muscle weight at ≥10 mg/kg/day; mild external genital dysgenesis, decreased reproductive organ weights, and Leydig cell hyperplasia at ≥300 mg/kg/day
Christiansen et al. 2010									
77	Rat (Wistar) 8–10 M	4 weeks (G)	0, 1,000, 5,000, 10,000	BH, BW, CS, FI, LE, HP, OF, OW, WI	Death Bd wt Cardio Hepatic Renal Endocr Immuno Neuro Repro	1,000	5,000	10,000 10,000	2/8 deaths due to emaciation 9% decrease in terminal body weight at 5,000 mg/kg/day; 32% decrease in terminal body weight at 10,000 mg/kg/day Decreased fertility, decreased testicular weight, severe atrophy of seminiferous tubules, and diffuse Leydig cell hyperplasia
Dalgaard et al. 2000									
78	Rat (Wistar) 10 M	9 weeks (GO)	0, 125, 250, 500, 1,000	BH, BW, CS, FI, HP, OW, WI	Bd wt Cardio Hepatic Renal Endocr Immuno Neuro Repro	1,000 1,000 1,000 1,000 1,000 1,000 1,000 1,000			
Dalgaard et al. 2000									

2. HEALTH EFFECTS

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) 10–12 F	42 days GD 1–PND 21 (GO)	0, 20, 100, 500	BW, DX, MX	Bd wt Repro Develop	500 100 20	100	500 500	Increased post-implantation loss, decreased litter size 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									
80	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 Hepatic Repro	1,093 1,093 1,093	2,496		35% decrease in body weight and 52% decrease in food consumption at 2,496 mg/kg/day Increased hepatocyte cytoplasmic eosinophilia Increased liver weight and peroxisome proliferation at ≥115 mg/kg/day ^b Decreased testes weight, bilateral testicular atrophy
Exxon Chemical Americas 1990									
81	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									
82	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, MX, DX, OW	Bd wt Renal Endocr Immuno Neuro Repro Develop	405 405 405 405 405 405 5	15		Delayed PPS and vaginal opening and decreased sperm 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									

2. HEALTH EFFECTS

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
83	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 ≥ 147 mg/kg/day; decreased 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									
84	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, MX, OF	Bd wt Repro Develop	300 300		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									

2. HEALTH EFFECTS

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
85	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 17% decrease in terminal body weight (38% decrease in body weight gain) with no significant changes in food consumption Decreased RBCs, hemoglobin, and hematocrit and increased platelets Decreased hemoglobin, hematocrit, segmented neutrophils, and myeloid/erythroid ratio Cellular pigmentation Increased liver weight at ≥ 62.7 mg/kg/day; hepatocellular enlargement at ≥ 261.2 mg/kg/day ^b Increased BUN at ≥ 261.2 mg/kg/day; increased kidney weight at ≥ 850.1 mg/kg/day; cellular pigmentation at 1,724 mg/kg/day Increased kidney weight and BUN at ≥ 918.4 mg/kg/day; cellular pigmentation at 1,857.6 mg/kg/day 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
						850.1 M	1,724 M		
					Resp	1,857.6			
					Cardio	1,857.6			
					Gastro	1,857.6			
					Hemato	261.2 M	850.1 M		
						918.4 F	1,857.6 F		
					Musc/skel	1,857.6			
					Hepatic	850.1	1,724		
					Renal	62.7 M	261.2 M		
						301.8 F	918.4 F		
					Ocular	1,857.6			
					Endocr	261.2	850.1		
					Immuno	1,857.6			
Neuro	1,857.6								

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

Figure key ^a	Species (strain)	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	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									
86	Rat (Sprague-Dawley) 8 F	36 days GD 6–PND 20 (GO)	0, 25, 100, 400	BW, DX	Bd wt Repro Develop	400 400 400			
Kobayashi et al. 2006									
87	Rat (Long-Evans) 8 M	21 days (GO)	0, 10, 750	BC, OF	Bd wt Repro	750	10		Increased serum LH, increased number and proliferation of Leydig cell precursors following elimination of mature Leydig cells using EDS
Li et al. 2012a									
88	Rat (Long-Evans) 8 M	35 days (GO)	0, 10, 750	BC, OF	Bd wt Repro	750	10		Decreased serum testosterone, increased number of Leydig cell precursors following elimination of mature Leydig cells using EDS
Li et al. 2012a									
89	Rat (Long-Evans) 2–6 F	19 days GDs 2–20 (GO)	0, 10, 100, 750	BW, DX, MX, OF	Bd wt Repro Develop	750 750	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									
90	Rat (Long-Evans) 11–13 F	31 days GD 12.5–PND 21.5 (GO)	0, 10, 750	BW, DX, OF	Bd wt Repro Develop	750 750	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									

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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
91	Rat (Wistar) 10–12 F	42 days GD 0–PND 21 (GO)	0, 1.25, 6.25	BC, BW, DX, MX	Endocr Repro Develop	6.25 6.25		1.25	≥10% decrease in body weight; decreased adipose tissue; pancreatic damage with impaired glucose homeostasis in adult offspring
Lin et al. 2011									
92	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									
93	Rat (Wistar) 20 M, 20 F	9 months (F)	0, 50, 200, 1,000	BI, BW, FI, HP, OW	Bd wt Hepatic	200	1,000 50		12–15% decreased body weight gain; no change in food consumption Morphological changes in bile ducts; increased liver weight, hepatocellular hypertrophy, enzyme induction
Mitchell et al. 1985									
94	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									
95	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									
96	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									

2. HEALTH EFFECTS

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 (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									
103	Rat (Sprague-Dawley) 12 F	16 days (GO)	0, 37.5, 75, 150, 300	BI, BW, HP, OW, OF	Bd wt Immuno	300 300			
Piepenbrink et al. 2005									
104	Rat (Sprague-Dawley) 12–13 F	16 days GDs 6–21 (GO)	0, 37.5, 75, 150, 300	DX, MX	Repro Develop	300	37.5		Increased AGD
Piepenbrink et al. 2005									
105	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 Resp Cardio Gastro Hemato Musc/skel Hepatic Renal Dermal Ocular Endocr Neuro	419.3 419.3 419.3 419.3 37.6 M 419.3 F 419.3 37.6 37.6 419.3 419.3 419.3 419.3	375.2 M 375.2 375.2		Decreased RBCs and hemoglobin Decreased serum cholesterol; increased liver weight, mild hypertrophy, and peroxisomal proliferation Increased kidney weight

2. HEALTH EFFECTS

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

Figure key ^a	Species (strain)	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	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									
106	Rat (Wistar) 6 M	30 days (G)	0, 10, 100	BC, BI	Other noncancer		10		Altered glucose metabolism/homeostasis
Rajesh et al. 2013									
107	Rat (Wistar) 10 M, 10 F	~19 weeks (2-generation) (F)	0, 130, 380, 1,040	BW, CS, DX, FI, HP, OF, OW	Death Bd wt Hepatic Renal Endocr Repro Develop	380 F 1,040 M 1,040 1,040 380 130	1,040 1,040	1,040	3/9 F1 males and 2/9 F1 females died Decreased F0 and F1 body weight and food consumption at 1,040 mg/kg/day Increased liver weights in adult females at ≥ 130 mg/kg/day and adult males at ≥ 380 mg/kg/day ^b Observed in one or both generations: decreased pups/dam, postimplantation loss, decreased reproductive organ weight, testicular lesions 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									
108	Rat (Wistar) 25 M, 25 F	19 weeks (2-generation) ~10 weeks prematuring–PND 21 (F)	0, 113, 340, 1,088	BH, BW, CS, FI, HP, OF, OW, LE	Death Bd wt Hepatic	340 113	340	1,088 F	6/25 deaths in F1 adult females Decreased body weight and food consumption in F0 females and adult F1 males and females at 1,088 mg/kg/day 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

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

Species Figure (strain) key ^a	Exposure No./group	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
				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		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
					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								
109	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								
110	Rat (Fischer- 344) 5 M	21 days (F)	0, 11, 105, 667, 1,223, 2,100	BI, BW	Bd wt			No weight gain during study at 2,100 mg/kg/day; food consumption not measured
Short et al. 1987								

2. HEALTH EFFECTS

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
111	Rat (Sprague-Dawley) 4 M	15 d 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									
112	Rat (Wistar) 10 F	42 days GD 0–PND 21 (GO)	0, 0.25, 6.25	BW, DX, MX	Repro Develop	6.25	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									
113	Rat (Wistar) 8 M	30 days (G)	0, 0.7, 70	HP, BI, OF	Immuno		0.7		Enhanced immune response to OVA challenge in sensitized 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									
114	Mouse (BALB/c) 8 M	52 days (G)	0, 0.03, 0.3, 3	BC, HP, OF	Immuno		0.03		Enhanced immune response to OVA challenge in sensitized animals
Guo et al. 2012									
115	Mouse (BALB/c) 4 M, 4 F	28 days (GO)	0, 0.03, 0.3, 3	OF	Immuno		0.03		Enhanced humoral immune response to OVA challenge in sensitized animals
Han et al. 2014									
116	Mouse (CD-1) 8 F	30 days (GO)	0, 0.02, 0.2, 20, 200	BW, OF, OW	Bd wt Repro	200 20		200	Increased percentage of days spent in estrus
Hannon et al. 2014									

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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	
117	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 7,899 F	4/10 males died 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	
					Hepatic	245	1,209		Slight to moderate focal coagulative necrosis and increased liver weight at $\geq 1,209$ mg/kg/day; increased hepatocellular hypertrophy at $\geq 2,579$ 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	2,579 M		Decreased testes weight at $\geq 2,579$ mg/kg/day; testicular atrophy and decreased spermatogenesis at 6,922 mg/kg/day						
	7,899 F									
Myers 1992a										
118	Mouse (A/J) 10 M	4 weeks (F)	0, 12.3, 125	BW, FI, HP, OF, WI	Bd wt	125				
					Repro		12.3			Sertoli cell vacuolation and germ cell sloughing in seminiferous tubules
Kitaoka et al. 2013										

2. HEALTH EFFECTS

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
119	Mouse (Cr1:CD-1) 20 M, 20 F	18 weeks (F)	0, 13, 130, 390	DX, FX, HP, MX, OF, OW	Bd wt Repro Develop	390 13 13	 130 130	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 6% decrease in female pup weight
Lamb et al. 1987; Morrissey et al. 1988; NTP 1984 [continuous breeding protocol with crossover mating]									
120	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	BW, CS, HP	Bd wt Resp Cardio Gastro Musc/skel Hepatic Renal Endocr Immuno Neuro Repro	 2,600 2,600 2,600 2,600 2,600 2,600 2,600 2,600 2,600			10–12% decrease in female body weight at ≥1,300 mg/kg/day and a 15% decrease in male body weight at 2,500 mg/kg/day; food consumption not reported
NTP 1982									
121	Mouse (CD-1) 7–10 F	42 days GD 0–PND 21 (F)	0, 0.05, 5, 500	DX, OW, OF	Repro Develop	5		500 0.05	Complete litter loss in 9/10 dams >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									
122	Mouse (CD-1) 28–29 F	18 days GDs 0–17 (F)	0, 19, 48, 95	BW, DX, FI, MX	Bd wt Repro Develop	95 48 48	 95	95	19% decrease in live pups/litter 11% decrease in postnatal viability from PND 1 to PND 4
Price et al. 1988b									

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

Figure key ^a	Species (strain)	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	Mouse (NC/Nga) 12 M	4 weeks 1 day/week (GO)	0, 0.0475, 0.095, 19	BC, CS, HP, OF	Immuno	19			
Sadakane et al. 2014 [mite-sensitized mice]									
124	Mouse (C57BL/6) 6 M	20 days (F)	0, 180, 360	BW, OW, OF	Bd wt Immuno	360 360			
Sasaki et al. 2003									
125	Mouse (C3H/N) 15F	8 weeks 7 weeks prematuring GD1 (F)	0, 0.05, 5, 500	BW, CS, FI, OF	Bd wt Repro Other noncancer	500	0.05 0.05		~20% increase in body weight 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									
126	Mouse (C3H/N) 15 F	8 weeks 7 weeks prematuring– GD 1 (F)	0, 0.05, 5, 500	BW, CS, FI, OF	Repro Develop Other noncancer	500		0.05 0.05	>20% increase in offspring body weight at PND 21, increased visceral adipose tissue 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									
127	Mouse (ICR) 7–12 F	18 days GDs 1–18 (F)	0, 85, 170, 341, 683, 1,707	BW, DX, FI, FX, MX, TG	Bd wt Repro Develop	170 170 170		341 341 341	26% decrease in maternal weight at GD 18; no change in food consumption 62.8% increase in resorptions and fetal mortality (combined); complete litter loss at ≥683 mg/kg/day 14–21% decrease in GD 18 fetal weight; 25.8 % increase in number of malformed fetuses
Shiota et al. 1980; Shiota and Nishimura 1982									

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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	
128	Mouse (CD-1) 10 M, 10 F	17 weeks 4 weeks prematuring– PNW 9 (F)	0, 20.62, 60.42, 180.77	BH, BW, DX, FI, OF, MX	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 TWA averages across sex and generation]										
129	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										
130	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										

2. HEALTH EFFECTS

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

Figure key ^a	Species (strain)	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
131	Mouse (CD-1) 24–25 F	17 days GDs 0–17 (F)	0, 44, 91, 191, 292	BW, CS, DX, GN, FI, MX, OW, TG, WI	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; decreased fetal weight at ≥191 mg/kg/day
Tyl et al. 1988									
132	Mouse (Sv/129) 15 M	24 weeks (F)	0, 2,400	CS, BW, HP, LE, OF, OW	Death			2,400	100% mortality between weeks 12 and 16
Ward et al. 1998									
133	Mouse (CD-1) 5 F	20 days GDs 0.5–18.5 (NS)	0, 0.04	BC, DX	Develop		0.04 ^d		Delayed meiotic progression of germ cells in GD 17.5 F1 fetuses; accelerated folliculogenesis in F1 and F2 PND 21 offspring
Zhang et al. 2015									
134	Guinea pig (NS) 4–5 M	15 days (GO)	0, 2,000	LE	Death			2,000	40% mortality
Parmar et al. 1988									
135	Rabbit (NS) NS M	15 days (GO)	0, 2,000	LE	Death			2,000	100% mortality
Parmar et al. 1988									
CHRONIC EXPOSURE									
136	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]									

2. HEALTH EFFECTS

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

Figure key ^a	Species (strain)	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
					Renal	36	147		Increased kidney weight at ≥ 147 mg/kg/day; increased severity of renal tubule pigmentation and 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									
140	Rat (Sprague-Dawley)	102 weeks (F)	0, 14, 140, 1,400	BW, CS, EA, HP	Bd wt				~8–10% decrease in body weight at 140 mg/kg/day and ~20–27% decreased in body weight at 1,400 mg/kg/day; food consumption was not measured
					Repro		14		“Inhibition” of spermatogenesis and general tubule atrophy (magnitude not reported)
					Cancer				No liver or testicular neoplasms (other organs not evaluated)
Ganning et al. 1991									
141	Rat (Fischer-344)	78 weeks (F)	0, 1,579	BW, HP, OW	Cancer			1,579	CEL: hepatocarcinomas
Hayashi et al. 1994									

2. HEALTH EFFECTS

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
142	Rat (Fischer-344) 50 M, 50 F	2 years (F)	M: 0, 322, 674 F: 0, 394, 774	BW, FI, HP, GN	Bd wt				11–14% decrease in male body weight at ≥322 mg/kg/day and 20% decrease in female body weight at 774 mg/kg/day; 14–15% decrease in food consumption at ≥322 mg/kg/day in both sexes
					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									
143	Rat (Fischer-344) NS M	365 days (F)	0, 930	BW FI OW HP BI	Bd wt				17% decrease in final body weight and 10% decrease in food consumption at 930 mg/kg/day
Marsman et al. 1988									
144	Rat (Wistar) NS	2 years (F)	0, 2,000	HP	Repro			2,000	Testicular atrophy
Price et al. 1987									

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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
145	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]									
146	Rat (Fischer-344) 10–14 M	108 weeks (F)	0, 1,600	BW, HP, OW	Bd wt Resp Gastro Renal Cancer	1,600	1,600 1,600	1,600	27% decrease in body weight at 1,600 mg/kg/day; food consumption not measured Pseudoductular lesions and altered acinar cell foci in the pancreas Lipofuscin pigments in tubular epithelium CEL: hepatocellular carcinoma, pancreatic islet-cell adenoma
Rao et al. 1990									
147	Rat (Wistar) 4 M	79 weeks (F)	0, 1,500	BI, BW, OW	Bd wt				21% decrease in body weight gain at 1,500 mg/kg/day; food consumption not measured
Tamura et al. 1990									
148	Rat (Sprague-Dawley) 60–390 M	Lifetime 6 days/week (F)	0, 30, 95, 300	BW, CS, HP, OW	Bd wt Resp Hepatic Endocr Immuno Neuro Repro Cancer	300 300 300 300 300 95	300	300	Seminiferous tubule atrophy CEL: Leydig cell tumors
Voss et al. 2005									

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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	
149	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		Hepatocyte pigmentation and 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										
150	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										
151	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									

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

Species Figure (strain) key ^a	Exposure No./group	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
				Renal	672 M 1,821 F	1,325 M		Chronic inflammation of the kidney
				Dermal	1,821			
				Endocr	1,821			
				Immuno	1,821			
				Neuro	1,821			
				Repro	672 M 799 F	1,821 F	1,325 M	Seminiferous tubular degeneration Suppurative inflammation in the uterus/endometrium
				Cancer			672	CEL: hepatocellular adenoma or carcinoma
Kluwe et al. 1982a, 1982b, 1985; NTP 1982								
152	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		
					Cancer			No exposure-related neoplasms
Carpenter et al. 1953 [female reproductive organs not assessed]								
153	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		
					Cancer			No exposure-related neoplasms
Carpenter et al. 1953								

2. HEALTH EFFECTS

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
154	Ferret (albino) 7 M	14 months (F)	0, 1,200	BI, BW, EA, OW, HP	Bd wt				31% decrease in body weight at 1,200; food consumption not measured
					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

^aThe 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.

^bHepatic 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.

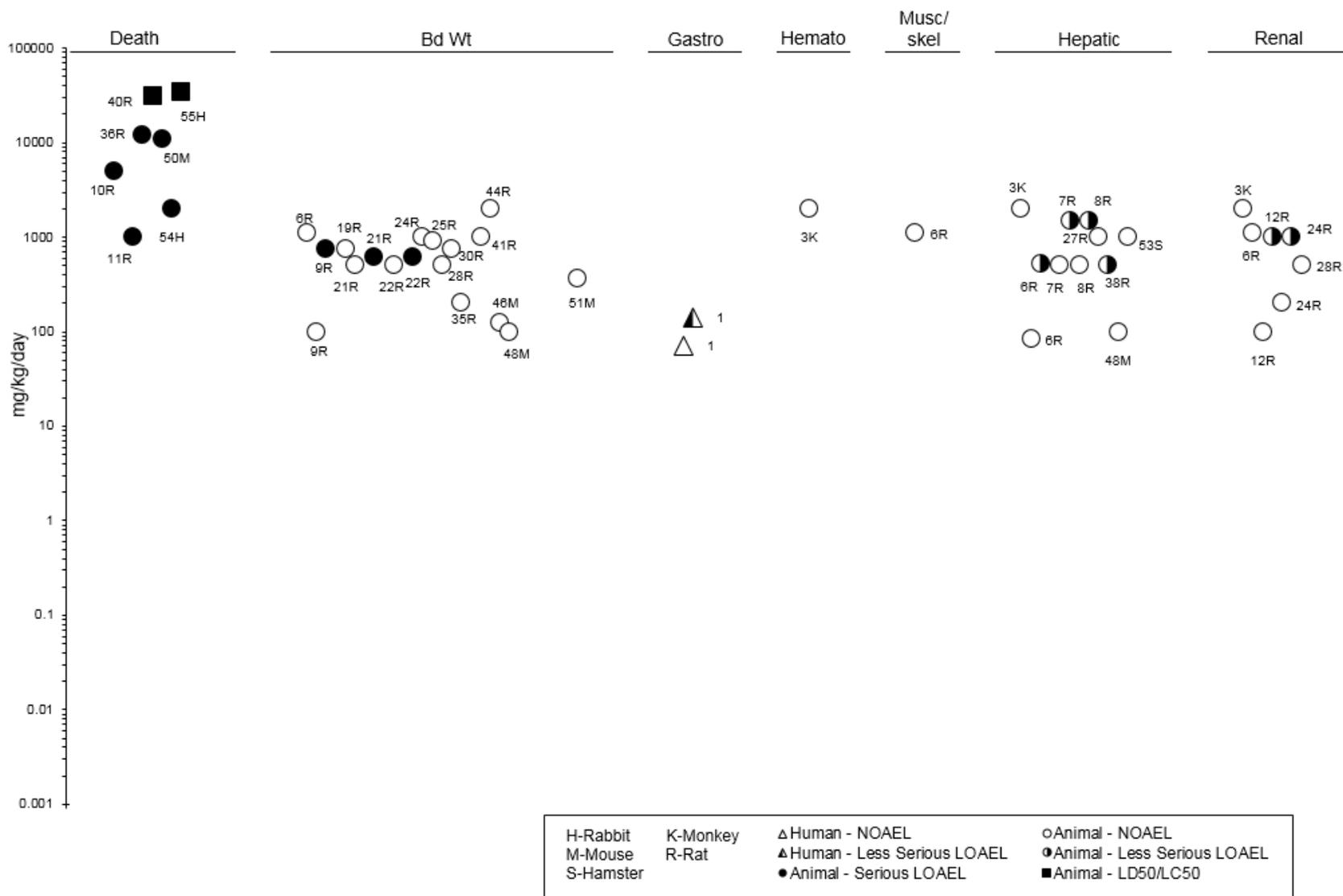
^cUsed to derive a provisional acute-duration oral minimal risk level (MRL). The LOAEL of 1 mg/kg/day was divided by an uncertainty factor of 300 (10 for use of a LOAEL; 3 for human variability, and 10 for animal to human extrapolation), resulting in a provisional MRL of 0.0003 mg/kg/day.

^dUsed to derive a provisional 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 a provisional MRL of 0.0001 mg/kg/day.

AGD = anogenital distance; AGI = anogenital index; B = both males and females (number per sex not reported); BC = serum (blood) chemistry; Bd Wt or BW = body weight; BH = behavioral; BI = biochemical changes; BM = blood metabolites; BUN = blood urea nitrogen; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Derm = dermal; 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; FX = fetal toxicity; (G) = gavage; Gastro = gastrointestinal; GD = gestational day; GN = gross necropsy; (GO) = gavage in oil; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LABC = levator ani/bulbocavernosus; LE = lethality; LH = luteinizing hormone; LD₅₀ = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; LSE = levels of significant exposure; M = male(s); Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; 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; TG = teratogenicity; TWA = time-weighted average; UR = urinalysis; (W) = drinking water; WI = water intake

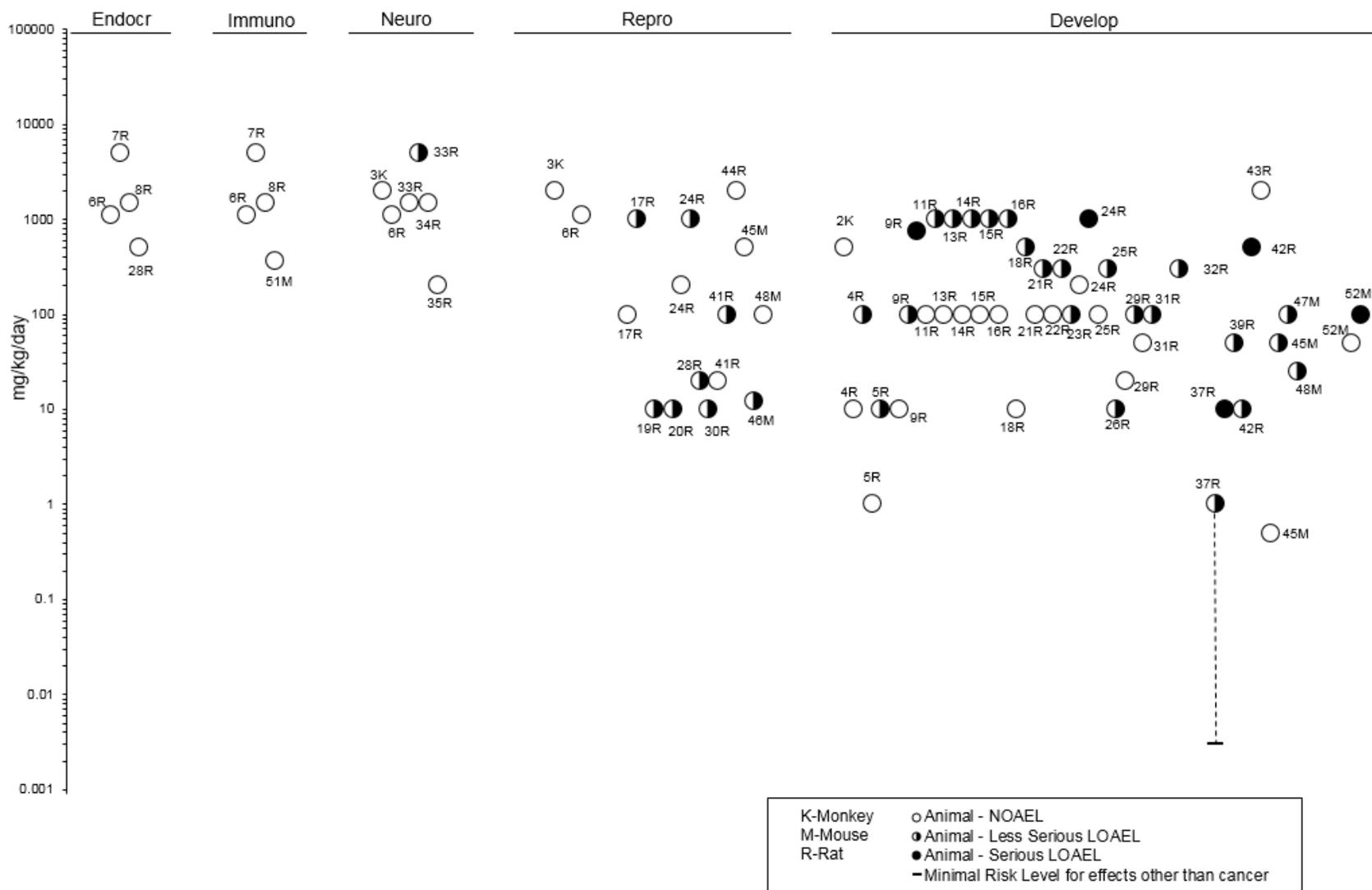
2. HEALTH EFFECTS

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



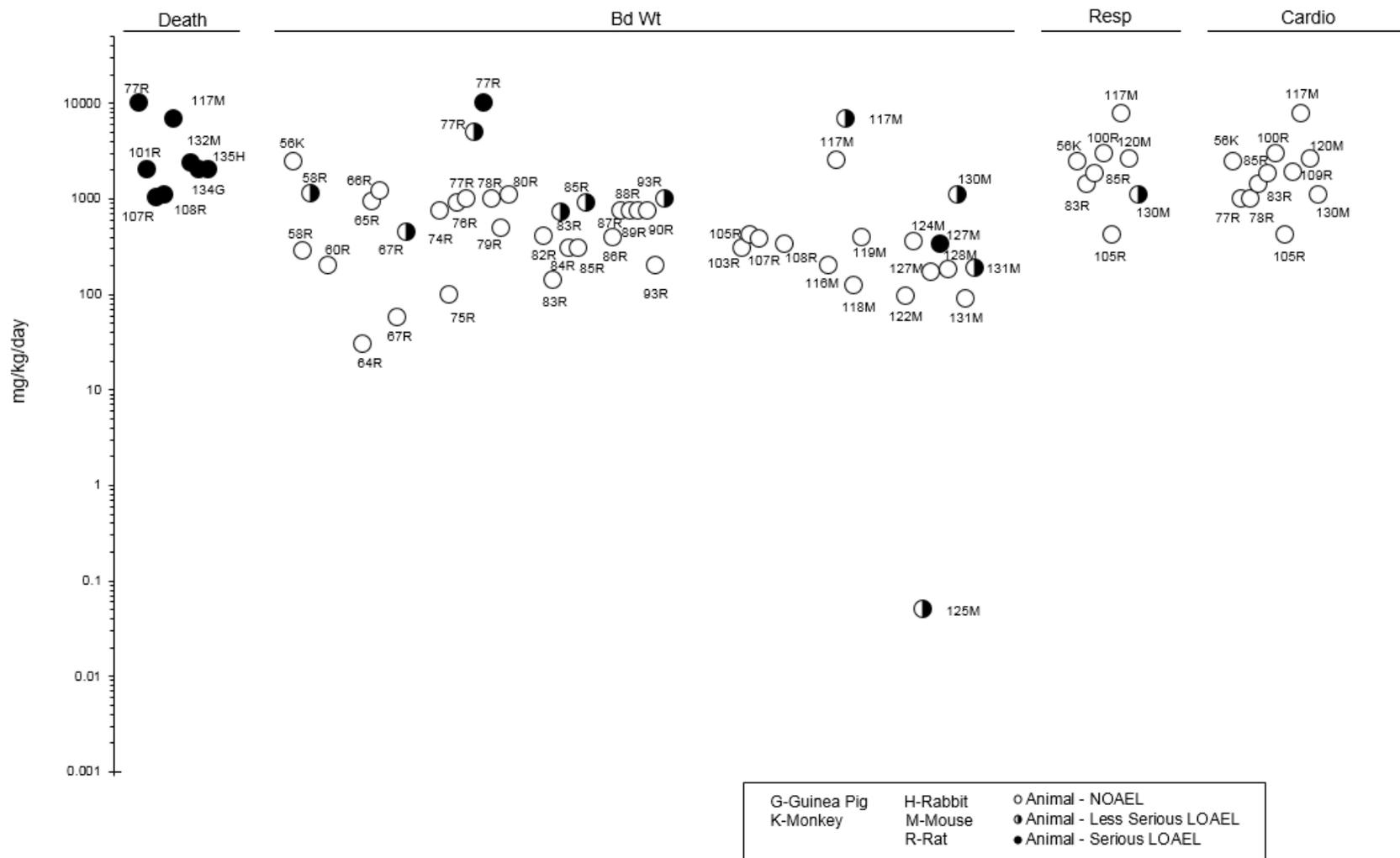
2. HEALTH EFFECTS

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



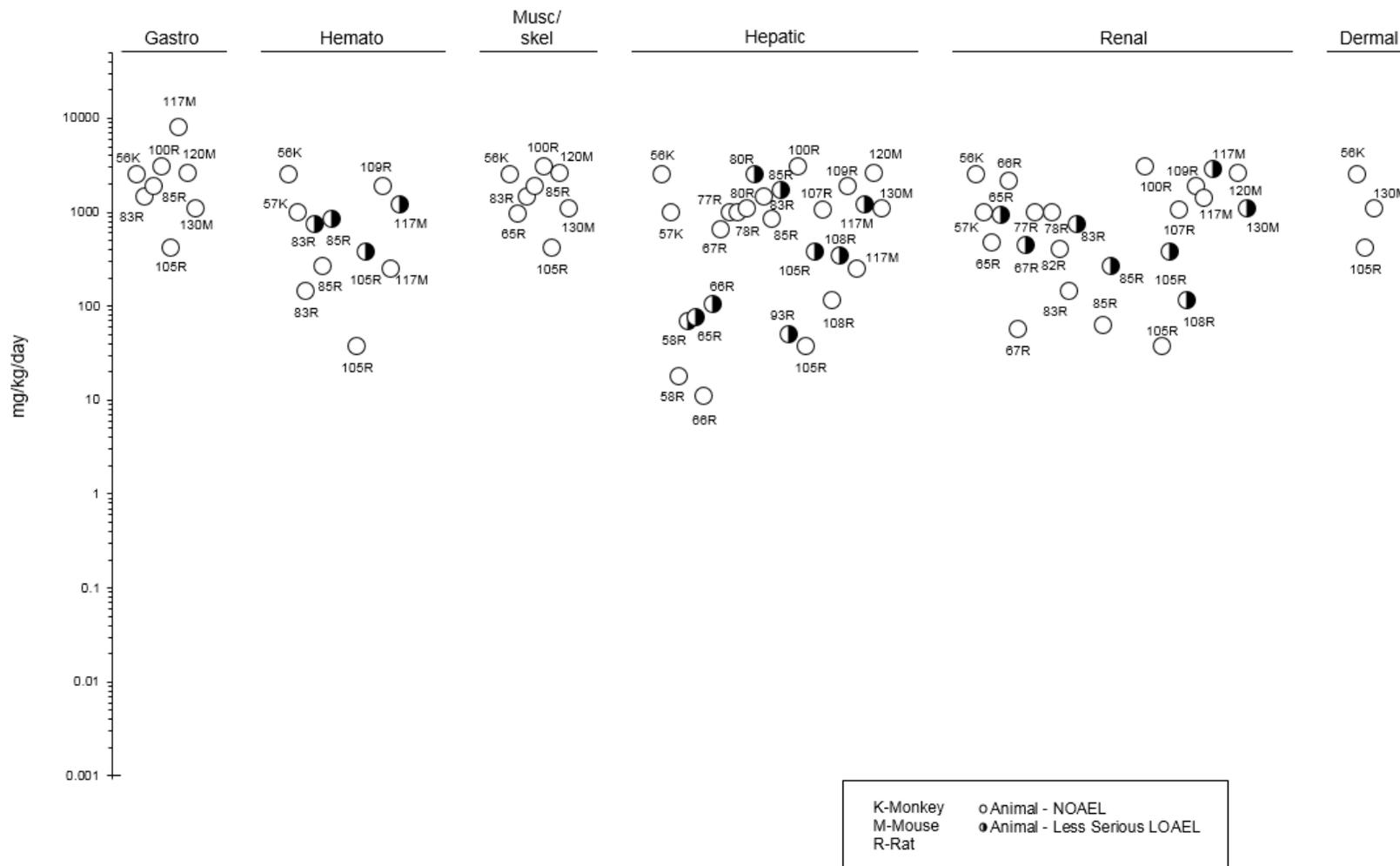
2. HEALTH EFFECTS

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



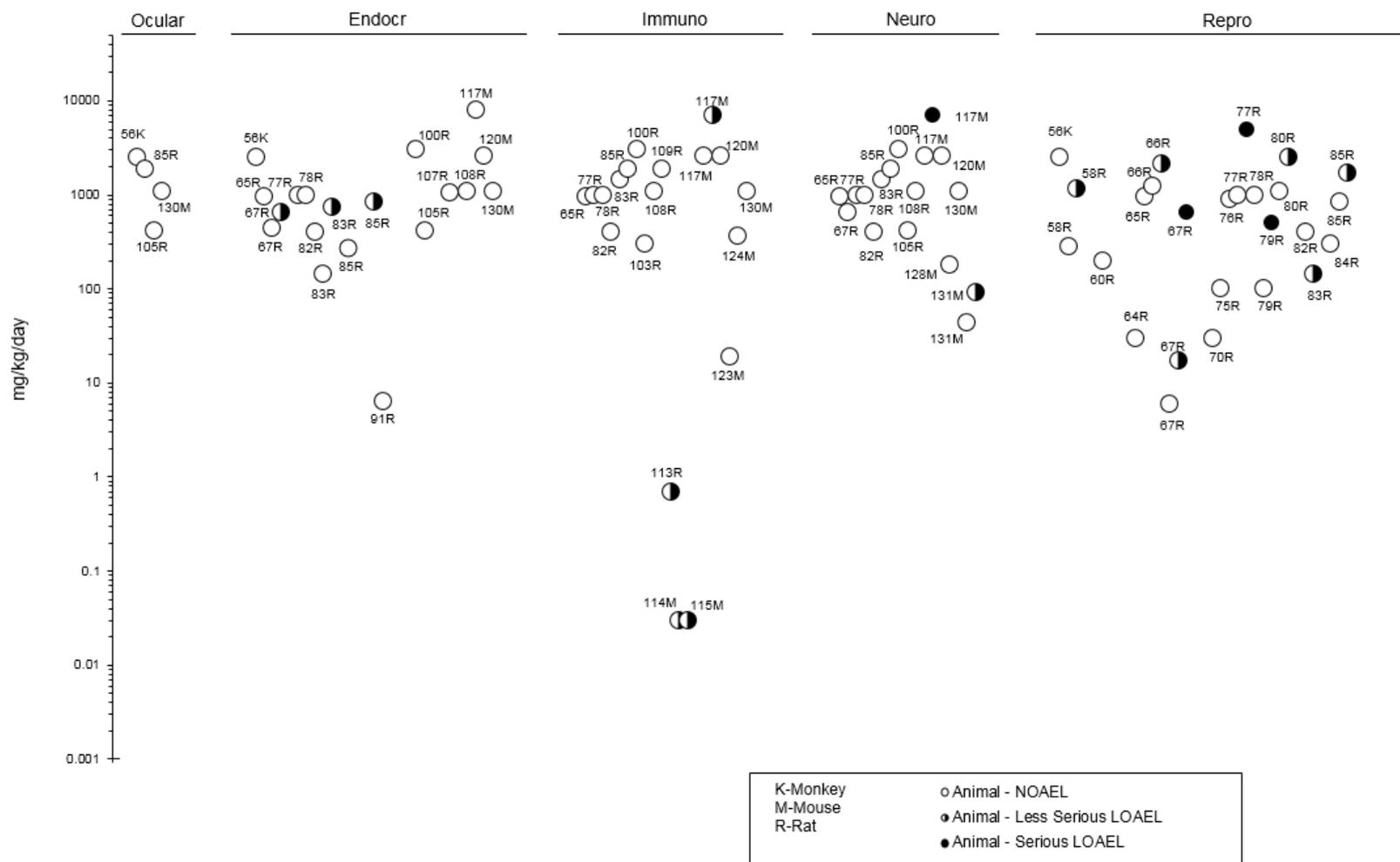
2. HEALTH EFFECTS

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



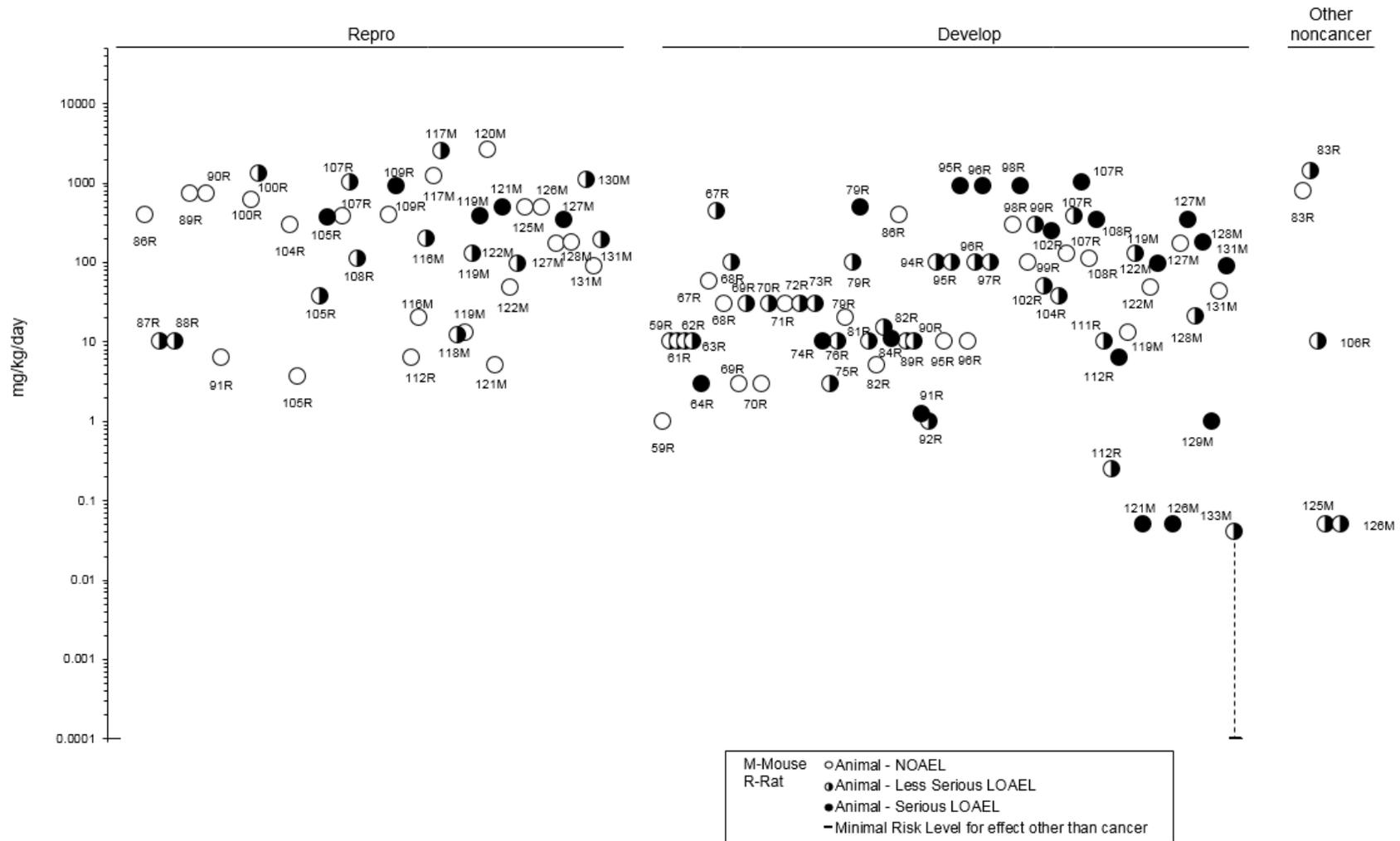
2. HEALTH EFFECTS

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



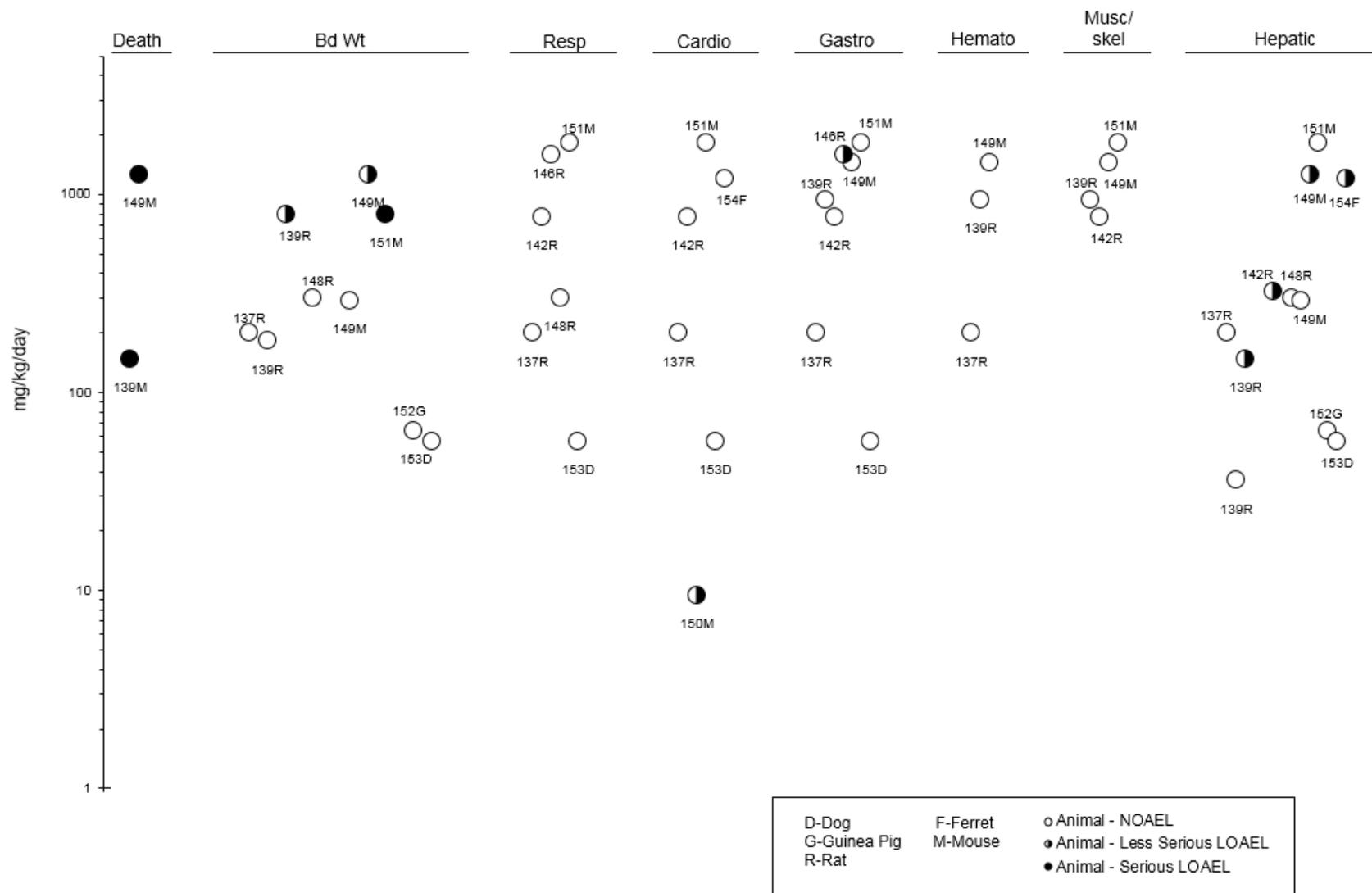
2. HEALTH EFFECTS

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



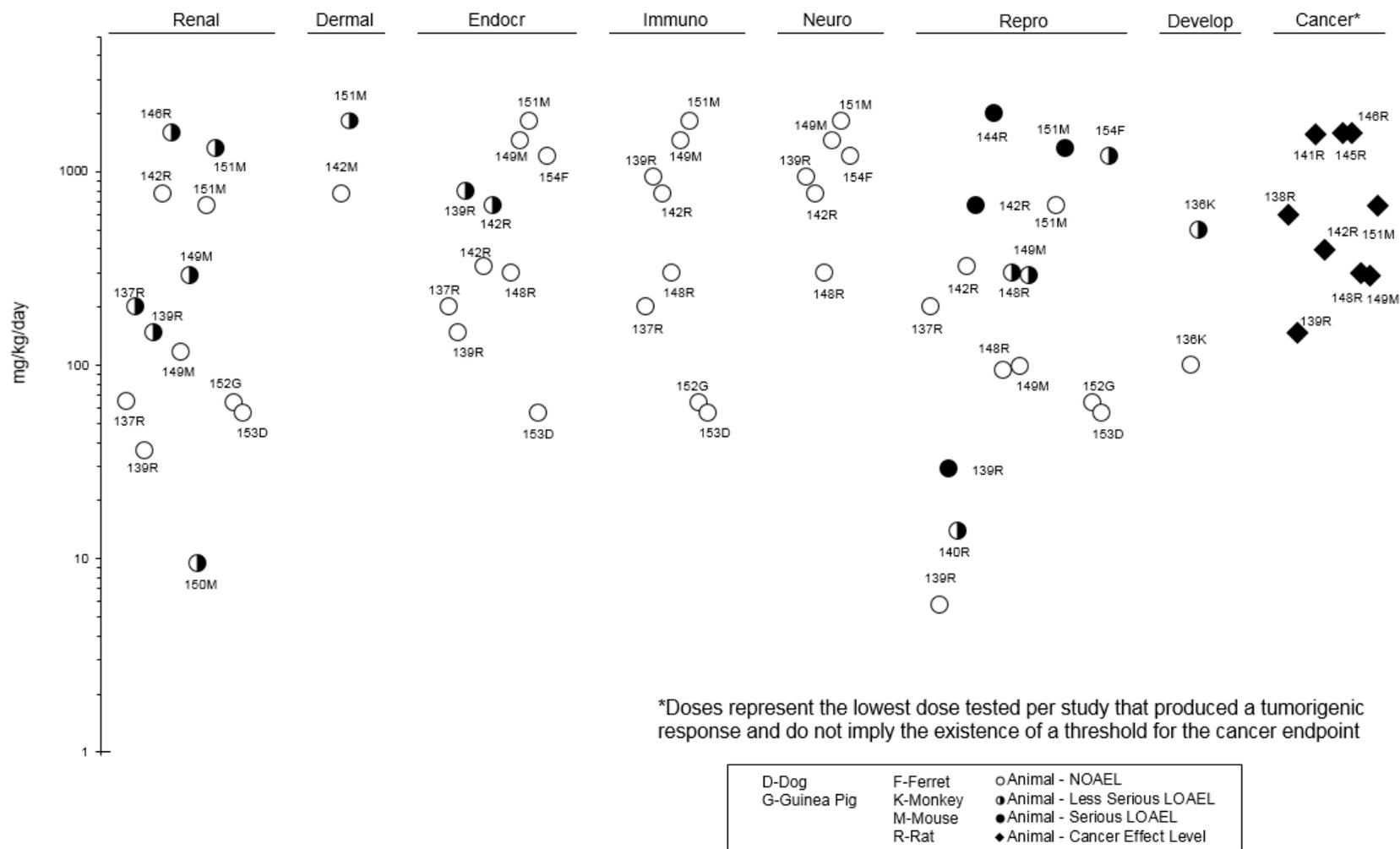
2. HEALTH EFFECTS

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



2. HEALTH EFFECTS

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



2. HEALTH EFFECTS

Table 2-3. Levels of Significant Exposure to DEHP – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
ACUTE EXPOSURE								
Human 23 NS	7 days	Undiluted	CS	Dermal	Undiluted			
Shaffer et al. 1945								
Rabbit (NS) 6 NS	Once	≤19,800 mg/kg	CS, LE	Death Dermal	19,800		19,800	2/6 died
Shaffer et al. 1945								

CS = clinical signs; DEHP = di(2-ethylhexyl)phthalate; LE = lethality; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; NS = not specified

2. HEALTH EFFECTS

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. On the other hand, DEHP was found to be lethal to rats after 2–4 hours of exposure to a mist prepared by passing air through a heated sample of DEHP (Shaffer et al. 1945). However, the concentration of DEHP in the mist was not measured.

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 LD₅₀ 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 \geq 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 \leq 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. 1998); 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.

2. HEALTH EFFECTS

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 $\geq 11,000$ mg/kg/day for 14 days (NTP 1982).

When rabbits were exposed to single dermal applications at doses up to 19,800 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 LD₅₀ value calculated from these data was 24,750 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 2-4; these include a cohort study (Teitelbaum et al. 2012) where exposure was measured approximately 1 year prior to anthropometric measurements; a cohort study (Bellavia et al. 2017) where exposure was measured in pregnant women during the first trimester and body weights were measured at first and second trimester visits; and nine cross-sectional or case-control studies that measured exposure and outcome at the same time. Six 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

2. HEALTH EFFECTS

Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Cohort studies				
Bellavia et al. 2017 Cohort (United States [Boston])	347 pregnant women with full-term births recruited from a prospective pregnancy cohort at Brigham and Women's Hospital (LIFECODES cohort), mean age 32 years; maternal urine samples collected at 1 st trimester visit (median 9.9 weeks), anthropometric measurements made at 1 st trimester visit (median 9.9 weeks) and 2 nd trimester visit (median 17.3 weeks) to determine early gestational weight gain (GWG).	Linear quantile regression adjusted for maternal age, race/ethnicity, education, smoking, alcohol, baseline BMI (at 1 st trimester visit), specific gravity (SG)	Change in GWG per log-unit increase in SG-adjusted urinary metabolite concentration (50 th percentile of GWG distribution) ΣDEHP 0.4 (0.2–0.8) µmol/L (GM [IQR]) (MEHP, MEHHP, MEOHP, MECPP)	Q1: Reference Q2: 0.23 (-0.53, 0.98) Q3: -0.98 (-1.74, -0.21) Q4: -0.42 (-1.22, 0.39)
Teitelbaum et al. 2012, Cohort (United States [New York])	379 Hispanic and Black children (299 girls, 80 boys; age 6–8 years) recruited at the Mount Sinai Medical Center Pediatric Clinic, local community health centers, and local schools during 2004–2008 for a prospective cohort study (Growing Up Healthy Study). Children's urine samples collected at baseline; anthropometric measurements made 1 year later (mean age 8.42 years at followup).	Linear regression adjusted for urinary creatinine, age, sex, sedentary hours, metabolic equivalent hours, Hispanic ethnicity, caloric intake, season in which urine sample was collected, and parental education	BMI change per natural log-unit increase in urinary metabolite concentration ΣDEHP Girls: 235.5 µg/g Cr (median); Boys: 251.2 µg/g Cr (median) MEHP Girls: 6.5 µg/g Cr; Boys: 6.3 µg/g Cr MEHHP Girls: 72.0 µg/g Cr; Boys: 75.7 µg/g Cr MEOHP Girls: 44.8 µg/g Cr; Boys: 50.4 µg/g Cr MECPP Girls: 114.2 µg/g Cr; Boys: 114.6 µg/g Cr	β -0.03 (-0.44, 0.39) β -0.03 (-0.38, 0.31) β 0.02 (-0.36, 0.41) β -0.04 (-0.43, 0.36) β -0.05 (-0.49, 0.38)
Analysis of BMI Z-score did not alter results.				

2. HEALTH EFFECTS

Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Change in waist circumference per natural log-unit increase in urinary metabolite concentration				
			ΣDEHP Girls: 235.5 µg/g Cr (median) Boys: 251.2 µg/g Cr (median)	β 0.00 (-1.11, 1.12)
			MEHP Girls: 6.5 µg/g Cr Boys: 6.3 µg/g Cr	β 0.06 (-0.85, 0.98)
			MEHHP Girls: 72.0 µg/g Cr Boys: 75.7 µg/g Cr	β 0.15 (-0.88, 1.19)
			MEOHP Girls: 44.8 µg/g Cr Boys: 50.4 µg/g Cr	β -0.01 (-1.07, 1.05)
			MECPP Girls: 114.2 µg/g Cr Boys: 114.6 µg/g Cr	β -0.12 (-1.28, 1.05)
Cross-sectional and case-control studies				
Bellavia et al. 2017	347 pregnant women with full-term births recruited from a prospective pregnancy cohort at Brigham and Women's Hospital that delivered between 2006 and 2008 (LIFECODES cohort), mean age 32 years; maternal urine samples and anthropometric measurements collected at 1 st trimester visit (median 9.9 weeks).	Linear quantile regression adjusted for maternal age, race/ethnicity, education, smoking, SG, and alcohol	Change in mean BMI per log-unit increase in SG-adjusted urinary metabolite concentration (entire cohort)	
Cross-sectional (United States [Boston])			ΣDEHP 0.4 (0.2–0.8) µmol/L (GM [IQR]) (MEHP, MEHHP, MEOHP, MECPP)	Q1: Reference Q2: 1.93 (0.34, 3.52) Q3: 1.2 (-0.41, 2.8) Q4: 1.5 (-0.17, 3.18)
			Change in 25 th percentile BMI per log-unit increase in SG-adjusted urinary metabolite concentration	
			ΣDEHP 0.4 (0.2–0.8) µmol/L (GM [IQR]) (MEHP, MEHHP, MEOHP, MECPP)	Q1: Reference Q2: 1.4 (0.12, 2.68) Q3: 1.23 (-0.06, 2.52) Q4: 2.32 (0.97, 3.67)

2. HEALTH EFFECTS

Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
			Change in 75 th percentile BMI per log-unit increase in SG-adjusted urinary metabolite concentration	
			ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	0.4 (0.2–0.8) μmol/L (GM [IQR])
				Q1: Reference Q2: 3.29 (1.09, 5.49) Q3: 2.68 (0.45, 4.9) Q4: 1.68 (-0.64, 4.01)
			No significant shift in BMI was observed at any exposure level for the 50 th percentile of BMI.	
James-Todd et al. 2016a	350 pregnant women with full-term births. All else the same as Bellavia et al. (2017).	Linear regression adjusted for maternal age, race/ethnicity, education, smoking, SG, and alcohol	Change in baseline BMI per log-unit increase in SG-adjusted urinary metabolite concentration	
Cross-sectional (United States [Boston])			ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	Q1: 0.12 μmol/L (median) Q2: 0.24 Q3: 0.53 Q4: 2.09
				Q1: 26.2 (24.0, 28.3) Q2: 27.3 (25.1, 29.5) Q3: 27.5 (25.2, 29.7) Q4: 27.2 (25.0, 29.4)
James-Todd et al. 2016b	965 cases of metabolic syndrome (464 men, 501 women) and 1,754 subjects without metabolic syndrome (924 men, 830 women), aged 20–80 years; participants in NHANES 2001–2010. Urine samples collected same day as anthropometric measurements.	Logistic regression adjusted for urinary creatinine, age, sex, race/ethnicity, total caloric intake, education, physical activity, smoking, and poverty	OR for central obesity (waist circumference ≥102 cm in men or ≥88 cm in women) comparing highest quartile of urinary concentration with lowest	
Case-control (United States)			ΣDEHP (MEHP, MEHHP, MEOHP)	With metabolic syndrome: 0.13 (0.12, 0.15) (GM [95% CI]) Without metabolic syndrome: 0.12 (0.10, 0.13)
				OR 1.66 (1.16, 2.36)*

2. HEALTH EFFECTS

Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Lin et al. 2016, Cross-sectional (Taiwan)	243 male and 550 female students (mean age 21.28 years), including 303 with and 486 without elevated blood pressure in childhood, from the YOTA study (recruited 1992–2000 from schools). Urine samples collected same day as anthropometric measurements.	Linear regression adjusted for age, gender, and smoking status	Association between BMI and log-transformed Cr-adjusted urinary metabolite concentration		
			MEHP	1.7–38.99 µg/g Cr	β 0.202* (NR)
			MEHHP	15.86–43.16 µg/g Cr	β 0.048 (NR)
			MEOHP	10.18–26.56 µg/g Cr	β -0.191 (NR)
Hou et al. 2015a, 2015b, Cross-sectional (Taiwan)	270 children and adolescents recruited from primary schools in Taipei, Taiwan (6.5–15 years), and 38 complainants involved in lawsuit regarding plasticizer-tainted foods (identified as part of Risk Assessment of Phthalate Incident in Taiwan program); ages 6.5–8 years. Urine samples collected same day as anthropometric measurements.	Linear and logistic regression adjusted for age, gender, and urinary creatinine	OR for increased BMI in highest quartile of urinary metabolite concentration compared with lowest		
			ΣDEHP	100.74–237.19	OR 1.48 (0.66, 3.3)
			MEHP	10.04–87.08	OR 0.78 (0.32, 1.9)
			MEHHP	23.49–60.30	OR 3.04 (1.25, 7.40)*
			MEOHP	16.43–41.00	OR 1.99 (0.79, 4.97)
			MECPP	31.70–77.63	OR 2.00 (0.82, 4.88)
			OR for increased waist-to-hip (circumference) ratio in highest quartile compared with lowest		
			ΣDEHP	100.74–237.19	OR 1.64 (0.82, 3.28)
			MEHP	10.04–87.08	OR 0.87 (0.42, 1.80)
			MEHHP	23.49–60.30	OR 2.44 (1.19, 5.01)*
			MEOHP	16.43–41.00	OR 2.90 (1.40, 6.03)*
			MECPP	31.70–77.63	OR 2.45 (1.19, 5.06)*

2. HEALTH EFFECTS

Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
			Change in waist circumference per unit increase in metabolite in the highest quartile compared to lowest	
			ΣDEHP 100.74–237.19	β 2.31 (-0.50, 5.12)
			MEHP 10.04–87.08	β -1.36 (-4.32, 1.60)
			MEHHP 23.49–60.30	β 4.18 (1.34, 7.03)*
			MEOHP 16.43–41.00	β 2.79 (-0.09, 5.68)
			MECPP 31.70–77.63	β 2.28 (-0.62, 5.17)
Yaghjian et al. 2015a, 2015b, Cross-sectional (United States)	6,005 women ≥18 years of age (not pregnant and not diabetic); participants in NHANES 1999–2004. Urine samples collected same day as anthropometric measurements.	Ordered logistic regression adjusted for age, race, education, poverty, total calories, total fat, physical activity, menopausal status/hormone use, alcohol consumption, and smoking	OR for increased BMI per interquartile increase in Cr-adjusted urinary metabolite levels	
			ΣDEHP 19.59–58.66 µg/g Cr	OR 0.93 (0.84, 1.03)
			MEHP 1.49–5.95 µg/g Cr	OR 1.12 (1.03, 1.23)*
			MEHHP 9.86–31.09 µg/g Cr	OR 0.90 (0.80, 1.00)
			MEOHP 6.83–19.84 µg/g Cr	OR 0.90 (0.80, 1.01)
			MECPP 17.16–49.78 µg/g Cr	OR 0.81 (0.66, 1.00)
			OR for increased WC per interquartile increase in Cr-adjusted urinary metabolite levels	
			ΣDEHP 19.59–58.66 µg/g Cr	OR 0.90 (0.81, 1.00)
			MEHP 1.49–5.95 µg/g Cr	OR 1.05 (0.96, 1.15)
			MEHHP 9.86–31.09 µg/g Cr	OR 0.88 (0.79, 1.00)
			MEOHP 6.83–19.84 µg/g Cr	OR 0.89 (0.78, 1.01)
			MECPP 17.16–49.78 µg/g Cr	OR 0.80 (0.64, 0.99)*

2. HEALTH EFFECTS

Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Buser et al. 2014, Cross-sectional (United States)	Children and adolescents (6–19 years) and non-pregnant, non-lactating adults (>19 years) from NHANES 2007–2008; subject number not reported. Urine samples collected same day as anthropometric measurements.	Logistic regression adjusted for age, race/ethnicity, calorie intake, serum cotinine, and urinary creatinine in all analyses, as well as income level (ages 6–19 only) and education level, recreational activity, smoking status, alcohol intake, and diabetes (adults only)	OR for obesity (BMI ≥30) in adults ≥20 years comparing highest quartile of urinary metabolite concentration with lowest quartile	
			ΣDEHP 0.18 (0.01) μmol/mL (GM [SE])	OR 1.62 (1.11, 2.37)*
			MEHP 2.01 (0.10)	OR 0.84 (0.55, 1.29)
			MEHHP 15.86 (0.85)	OR 1.51 (1.07, 2.14)*
			MEOHP 9.16 (0.47)	OR 1.44 (1.02, 2.05)*
			MECPP 24.30 (1.20)	OR 1.85 (1.29, 2.64)*
			OR for overweight (BMI 25–29.9) in adults ≥20 years comparing highest quartile of urinary metabolite concentration with lowest quartile	
			ΣDEHP 0.18 (0.01) μmol/mL (GM [SE])	OR 1.22 (0.89, 1.67)
			MEHP 2.01 (0.10)	OR 1.02 (0.78, 1.34)
			MEHHP 15.86 (0.85)	OR 1.15 (0.87, 1.53)
			MEOHP 9.16 (0.47)	OR 1.28 (0.98, 1.69)
			MECPP 24.30 (1.20)	OR 1.26 (0.87, 1.84)
			OR for obesity (BMI z-score ≥95 th percentile) in children and adolescents (6–19) comparing highest quartile of urinary metabolite concentration with lowest quartile	
			ΣDEHP 0.24 (0.01) μmol/mL (GM [SE])	OR 1.09 (0.48, 5.49)
			MEHP 2.18 (0.11)	OR 0.84 (0.39, 1.80)
			MEHHP 21.03 (1.25)	OR 1.08 (0.51, 2.29)
MEOHP 12.92 (0.72)	OR 1.07 (0.45, 2.58)			
MECPP 34.79 (1.66)	OR 0.96 (0.41, 2.24)			

2. HEALTH EFFECTS

Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
			OR for overweight (BMI z-score between the 85 th and 95 th percentile) in children and adolescents (6–19 years old) comparing highest quartile of urinary metabolite concentration with lowest quartile	
			ΣDEHP 0.24 (0.01) μmol/mL (GM [SE])	OR 1.02 (0.44, 2.39)
			MEHP 2.18 (0.11)	OR 0.84 (0.41, 1.72)
			MEHHP 21.03 (1.25)	OR 1.11 (0.50, 2.50)
			MEOHP 12.92 (0.72)	OR 1.35 (0.59, 3.08)
			MECPP 34.79 (1.66)	OR 1.11 (0.54, 2.30)
Song et al. 2014, Cohort (United States [NHANES])	977 non-diabetic nurses from the Nurses' Health Study (NHS; age 30–55 years) and NHSII (age 25–42) recruited during 1996–2002 and followed for 10 years.		Association between BMI or weight gain and urinary metabolite concentration. ΣDEHP (MEHP, MEHHP, MEOHP, MECPP) 115–870 nmol/L	Effect estimates not reported; no significant association between metabolite levels and BMI or body weight gain.
Zhang et al. 2014, Cross-sectional (China)	493 children (247 boys, 246 girls, ages 8–13 years) recruited from suburban district in Shanghai between October and November 2011 (for the Puberty Timing and Health Effects in Chinese Children study). Urine samples collected same day as anthropometric measurements.	Logistic regression adjusted for socio-economic level, physical activity, dietary nutrient intake, puberty onset, and phthalate metabolite concentrations.	OR for obesity (weight >90 th percentile) comparing highest and lowest quartiles of log-transformed urinary metabolite concentrations in girls	
			ΣDEHP Girls 8–10 years: 5.2–497.7 Girls 11–13 years: 1.3–864.4 (min–max)	OR 0.078 (0.008, 0.791)*
			MEHP Girls 8–10 years: <LOD–92.2 Girls 11–13 years: <LOD–117.1 (min–max)	OR 0.128 (0.013, 1.242)
			MEHHP Girls 8–10 years: 3.2–290.0 Girls 11–13 years: 0.8–508.4 (min–max)	OR 0.084 (0.008, 0.91)*
			MEOHP Girls 8–10 years: 1.2–115.5 Girls 11–13 years: <LOD–238.8 (min–max)	OR 0.092 (0.009, 0.958)*

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Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
OR for overweight (weight >80 th and <90 th percentiles) comparing highest and lowest quartiles of log-transformed urinary metabolite concentrations in girls				
			ΣDEHP Girls 8–10 years: 5.2–497.7 Girls 11–13 years: 1.3–864.4 (min–max)	OR 0.811 (0.273, 2.405)
			MEHP Girls 8–10 years: <LOD–92.2 Girls 11–13 years: <LOD–117.1 (min–max)	OR 0.664 (0.230, 1.913)
			MEHHP Girls 8–10 years: 3.2–290.0 Girls 11–13 years: 0.8–508.4 (min–max)	OR 1.047 (0.339, 3.232)
			MEOHP Girls 8–10 years: 1.2–115.5 Girls 11–13 years: <LOD–238.8 (min–max)	OR 0.092 (0.305, 2.829)
Results for boys were not reported.				
Dirtu et al. 2013, Case-control (Belgium)	152 obese individuals recruited at the entry of a 12-month weight-loss program between November 2009 and February 2012 (46 men, 106 women; aged 18–84 years) and 43 non-obese, age- and sex-matched controls (12 men, 30 women; aged 19–59 years). Urine samples collected and anthropometric measurements made at baseline.	Linear regression adjusted for age and gender	Association between WC and urinary metabolite concentration in non-obese controls	
			ΣDEHP Controls: 27–53	β -0.24
			MEHP Controls: 2–5	β -0.10
			MEHHP Controls: 9–19	β -0.20
			MEOHP Controls: 3–9	β -0.29*
			MECPP Controls: 12–20	β -0.26*
Association between waist circumference at baseline and urinary metabolite concentration in obese cases				
			ΣDEHP Cases: 30–61	β 0.01
			MEHP Cases: 2–5	β -0.05
			MEHHP Cases: 10–25	β 0.01
			MEOHP Cases: 4–11	β -0.04
			MECPP Cases: 12–22	β 0.04

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Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a		
Wang et al. 2013, Cross-sectional (China)	259 students (ages 8–15 years) randomly selected from three primary and three middle schools in the Changning District of Shanghai City in 2011–2012; 124 normal weight, 53 overweight, and 82 obese subjects. Urine samples may have been collected after anthropometric measurements.	Linear regression adjusted for age, sex, and sum of other phthalates	Association between BMI and ln-transformed urinary metabolite concentrations			
			ΣDEHP 117.3 (GM)	β 0.015 (-0.026, 0.056)		
			MEHP 21.3	β 0.048 (0.007, 0.089)*		
			MEHHP 16.1	β 0.001 (-0.035, 0.037)		
			MEOHP 22.9	β -0.001 (-0.037, 0.036)		
			MECPP 28.8	β -0.006 (-0.042, 0.029)		
			Association between waist circumference and ln-transformed urinary metabolite concentrations			
			ΣDEHP 117.3 (GM)	β 0.012 (-0.021, 0.044)		
			MEHP 21.3	β 0.038 (0.006, 0.071)*		
			MEHHP 16.1	β -0.002 (-0.03, 0.027)		
					MEOHP 22.9	β 0.001 (-0.028, 0.03)
					MECPP 28.8	β -0.007 (-0.035, 0.022)
Hatch et al. 2008, Cross-sectional (United States [NHANES])	2,118 females and 2,251 males (age 6–80) from the general population; NHANES 1999–2002.	Linear regression adjusted for age, creatinine, height, race/ethnicity, socioeconomic status, percent of daily calories from total fat, daily servings of dairy, fruits, and vegetables, and television/video/computer use	Difference in BMI or WC between highest and lowest quartiles of urinary phthalate metabolite concentrations: females age 6–11 years			
			MEHP 5.4 (2.8) µg/g Cr (GM [SD])	BMI β 0.90 (-2.51, 0.71) WC β -2.51 (-6.52, 1.49)		
			MEHHP 39.6 (2.5) µg/g Cr	BMI β 0.54 (-1.50, 2.57) WC β 1.83 (-3.48, 7.13)		
			MEOHP 27.5 (2.4) µg/g Cr	BMI β -0.17 (-2.60, 2.26) WC β 0.45 (-5.56, 6.46)		
			Difference in BMI or WC between highest and lowest quartiles of urinary phthalate metabolite concentrations: females age 12–19 years			
			MEHP 3.8 (2.9) µg/g Cr (GM [SD])	BMI β -2.18 (-4.99, 0.63) WC β -1.51 (-2.81, -0.21)*		

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Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
			MEHHP 21.1 (2.6) µg/g Cr	BMI β 0.74 (-1.18, 2.65) WC β 1.81 (-3.19, 6.83)
			MEOHP 15.0 (2.4) µg/g Cr	BMI β 0.89 (-1.40, 3.18) WC β 1.79 (-4.10, 7.68)
Difference in BMI or WC between highest and lowest quartiles of urinary phthalate metabolite concentrations: females age 20–59 years				
			MEHP 4.0 (2.9) µg/g Cr (GM [SD])	BMI β -1.68 (-3.57, 0.21) WC β -2.17 (-5.99, 1.65)
			MEHHP 18.3 (2.8) µg/g Cr	BMI β 1.08 (-0.75, 2.92) WC β 3.13 (-0.73, 6.99)
			MEOHP 12.5 (2.7) µg/g Cr	BMI β 0.38 (-1.90, 2.66) WC β 1.52 (-2.98, 6.02)
Difference in BMI or WC between highest and lowest quartiles of urinary phthalate metabolite concentrations: females age 60–80 years				
			MEHP 3.3 (2.9) µg/g Cr (GM [SD])	BMI β -2.07 (-3.42, -0.73)* WC β -4.15 (-7.48, -0.81)*
			MEHHP 18.4 (2.7) µg/g Cr	BMI β -0.96 (-4.04, 2.11) WC β -2.82 (-8.89, 3.25)
			MEOHP 12.4 (2.6) µg/g Cr	BMI β 0.94 (-2.98, 4.85) WC β 2.46 (-7.41, 12.32)
Difference in BMI or WC between highest and lowest quartiles of urinary phthalate metabolite concentrations: males age 6–11 years				
			MEHP 5.5 (3.1) µg/g Cr (GM [SD])	BMI β -0.22 (-1.32, 0.89) WC β 0.55 (-3.31, 4.4)
			MEHHP 39.1 (2.4) µg/g Cr	BMI β 0.42 (-1.09, 1.92) WC β 1.27 (-2.43, 4.96)
			MEOHP 26.6 (2.4) µg/g Cr	BMI β 0.14 (-1.21, 1.48) WC β 0.6 (-2.68, 3.88)

2. HEALTH EFFECTS

Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Difference in BMI or WC between highest and lowest quartiles of urinary phthalate metabolite concentrations: males age 12–19 years				
			MEHP 2.7 (3.0) µg/g Cr (GM [SD])	BMI β -0.50 (-1.95, 0.94) WC β -1.39 (-5.15, 2.37)
			MEHHP 18.2 (2.8) µg/g Cr	BMI β 1.00 (-0.69, 2.69) WC β 2.15 (-1.77, 6.08)
			MEOHP 12.2 (2.8) µg/g Cr	BMI β 0.27 (-1.4, 1.94) WC β 0.68 (-2.67, 4.02)
Difference in BMI or WC between highest and lowest quartiles of urinary phthalate metabolite concentrations: males age 20–59 years				
			MEHP 3.3 (3.2) µg/g Cr (GM [SD])	BMI β 0.44 (-0.62, 1.52) WC β 0.91 (-1.43, 3.24)
			MEHHP 16.6 (3.0) µg/g Cr	BMI β 1.74 (-0.28, 3.76) WC β 4.60 (-0.03, 9.24)
			MEOHP 10.6 (2.8) µg/g Cr	BMI β 2.14 (-0.13, 4.41) WC β 5.81 (0.69, 10.94)
Difference in BMI or WC between highest and lowest quartiles of urinary phthalate metabolite concentrations: males age 60–80 years				
			MEHP 2.5 (2.9) µg/g Cr (GM [SD])	BMI β -1.16 (-2.60, 0.28) WC β -2.42 (-5.76, 0.93)
			MEHHP 13.2 (2.9) µg/g Cr	BMI β 0.41 (-2.47, 3.28) WC β 0.68 (-7.42, 8.78)
			MEOHP 9.2 (2.7) µg/g Cr	BMI β 0.69 (-2.05, 3.44) WC β 2.31 (-4.97, 9.59)

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Table 2-4. Selected Epidemiological Studies of DEHP Exposure and Body Weight Metrics

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Stahlhut et al. 2007, Cross-sectional (United States [NHANES])	1,451 adult males >18 years who were not taking insulin, oral hypoglycemic agents, or sex hormone agonists/ antagonists; participants in NHANES 1999–2002. Urine samples collected same day as anthropometric measurements.	Linear regression adjusted for age, age, race/ethnicity, total fat and calorie intake, physical activity level, smoking exposure, urinary creatinine, GFR, ALT, and GGT	Association between waist circumference and log-transformed urinary metabolite concentration		
			MEHP	11±1.3 µg/g Cr (mean±SE)	β 0.53 (NR)
			MEHHP	65.8±7.9 µg/g Cr	β 1.65* (NR)
			MEOHP	38.7±4.5 µg/g Cr	β 1.79* (NR)

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

ΣDEHP = sum DEHP metabolites; ALT = alanine transaminase transferase; BMI = body mass index; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GFR = glomerular filtration rate; GGT = gamma-glutamyl transferase; GM = geometric mean; IQR = interquartile range; LOD = limit of detection; max = maximum; MECPP = 2-ethyl-5-carboxypentylphthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl)phthalate; min = minimum; NHANES = National Health and Nutrition Examination Survey; NR = not reported; OR = odds ratio; SD = standard deviation; SE = standard error; SG = specific gravity; WC = waist circumference; YOTA = Young Taiwanese Cohort

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development. Teitelbaum et al. (2012) observed no association between DEHP metabolite levels in the urine of 7-year-old children and BMI or waist circumference in the children at age 8 years.

Bellavia et al. (2017) observed an inverse U-shaped relationship between first trimester urinary Σ DEHP metabolite levels and early gestational weight gain (between first and second trimesters) in a cohort of pregnant women with full-term births in a prospective analysis. In a cross-sectional analysis of the same cohort, urinary Σ DEHP metabolite levels were associated with higher first trimester BMI (Bellavia et al. 2017). In other cross-sectional and case-control studies, associations were reported for increased BMI in adults (Lin et al. 2016; Yaghjyan et al. 2015a, 2015b) and children (Hou et al. 2015a, 2015b; Wang et al. 2013), waist circumference in children (Hou et al. 2015a, 2015b; Wang et al. 2013), and increased odds of central obesity (waist circumference ≥ 102 cm in men or ≥ 88 cm in women) and obesity (BMI ≥ 30) in adults (Buser et al. 2014; James-Todd et al. 2016b). 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 $>90^{\text{th}}$ percentile) in children aged 8–13 years; and Dirtu et al. (2013) reported negative associations between waist circumference and DEHP metabolite levels.

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. (2016b), 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 (Lin et al. 2016; Yaghjyan et al. 2015a, 2015b) or reported results after adjustment for urinary creatinine (Buser et al. 2014; Hou et al. 2015a, 2015b;

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James-Todd et al. 2016b; 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 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. No exposure-related changes in body weight have been reported in nonpregnant, adult rodents following gavage exposure to acute doses $\leq 2,000$ mg/kg/day (Guo et al. 2013; Lee and Koo 2007; Li et al. 2012a; Moser et al. 2003; Stroheker et al. 2005; Zacharewski et al. 1998) or intermediate-duration doses $\leq 1,000$ mg (Akingbemi et al. 2001; Dalgaard et al. 2000; Hannon et al. 2014; Li et al. 2012a; Piepenbrink et al. 2005). 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). 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

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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 12 additional rodent studies evaluating exposure during gestation/lactation at gavage doses $\leq 1,000$ mg/kg/day (Table 2-2).

Dietary studies in rodents. Acute dietary studies do not report body weight effects at doses $\leq 1,250$ mg/kg/day in rodents (Astill et al. 1986; Kitaoka et al. 2013; Muhlenkamp and Gill 1998; Sasaki et al. 2003). A 17% decrease was reported in mice following dietary exposure to 3,850 mg/kg/day for 7 days (Muhlenkamp and Gill, 1998); however, food consumption was not measured.

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 1984; 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 studies without food consumption data, body weight effects in rats were observed at doses $\geq 2,100$ mg/kg/day, but not $\leq 1,300$ mg/kg/day (Agarwal et al. 1986; Mitchell et al. 1985; NTP 1982; Short et al. 1987).

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. 2011). 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 studies without food consumption data, body weight effects in mice were observed at doses $\geq 1,300$ mg/kg/day, but not $\leq 1,200$ mg/kg/day (NTP 1982; Sasaki et al. 2003).

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). However, other 1- to 2-year studies in F344 rats reported both reduced body weights and reduced food intake levels

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at dietary doses ≥ 322 mg/kg/day (Kluwe et al. 1982a; Marsman et al. 1988; NTP 1982). In chronic rat studies without food consumption data, body weight effects in rats were generally observed at doses >300 mg/kg/day (Carpenter et al. 1953; Rao et al. 1990; Tamura et al. 1990; Voss et al. 2005). However, one study in Sprague-Dawley rats reported an approximate 10 and 20% decrease in body weight after 6 months of exposure to 140 and 1,400 mg/kg/day, respectively, with terminal body weight decreases of approximately 8 and 27%, respectively, after 102 weeks (Ganning et al. 1991). 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 (Price et al. 1988b; 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.

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).

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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 PPAR α ; (2) promotion of differentiation and lipid accumulation in 3T3-L1 cells (embryonic mouse fibroblasts that differentiate to adipocyte-like cells) by mono(2-ethylhexyl)phthalate (MEHP), a PPAR γ 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).

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. One study reported elevated body weight with low dietary exposure (Schmidt et al.2012); additional endpoints from this study 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. In a panel study with repeated urine samples and spirometry tests in 418 Korean adults >60 years old, increased DEHP metabolite (mono-2-ethyl-5-hydroxyhexylphthalate [MEHHP] and mono-(2-ethyl-5-oxohexyl)phthalate [MEOHP]) levels in urine were associated with poorer pulmonary function test scores (forced expiratory volume in 1 second [FEV₁]/forced vital capacity [FVC] and forced expiratory flow at 25–75% of FVC [FEF_{25–75}]; Park et al. 2013). In this study (Park et al. 2013), the

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authors observed altered associations when the data were stratified by genetic polymorphisms in catalase (CAT), superoxide dismutase (SOD2), and myeloperoxidase (MPO), suggesting that gene-environment interactions may alter the effect of DEHP exposure on lung function. A 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 increase in the sum of DEHP metabolites (MEHP, MEHHP, MEOHP) in the urine was associated with impaired lung function (FEV₁, FVC, and FEV₁/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 III, 1988–1994 (Hoppin et al. 2004). Kolena et al. (2014) observed *improved* pulmonary function (FEV₁/FVC) with higher urinary MEHP levels (mean 15 ng/mL) in a study of 30 community service workers (mean age 46 years) with exposure to DEHP along with other air pollutants for an average of 7.9 years (men) and 5.6 years (women) during waste processing or loading; other DEHP metabolites were not evaluated. Interpretation of this study is limited by small sample size.

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 (decreased tidal volume and increased respiratory rate) was observed during lung function analysis of female mice following a 60-minute exposure to DEHP at 19 ppm (Larsen et al. 2007). No alterations in lung function were observed 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.

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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 (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 PND 1 and 21 offspring of rats exposed to DEHP at gavage doses of 750 mg/kg/day from GD 12 to PND 0 or from GD 12 to PND 21, respectively (Chen et al. 2010). Lung alterations included increased thickness of alveolar septa and less airspace in the lung, 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 maternal doses \leq 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. 2014; Larsen et al. 2007; 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.

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2.5 CARDIOVASCULAR

Overview. Available epidemiological studies evaluating cardiovascular effects (that met selection criteria) include cross-sectional and case-control studies of blood pressure. 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 one cohort study in pregnant women, and five cross-sectional studies in the general population (Table 2-5). Four of the five cross-sectional studies (James-Todd et al. 2016b; Shiue and Hristova 2014; Trasande and Attina 2015; Trasande et al. 2013b) used NHANES data and reported associations between DEHP urinary metabolite levels and increased blood pressure. 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 cohort, no associations were observed between maternal blood pressure or pregnancy-induced hypertensive disorders and DEHP metabolite concentration in maternal urine (Werner et al. 2015).

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.

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; however, these effects are likely secondary to the observed renal dysfunction in this study, as discussed in Section 2.10 (Renal). Elevated blood pressure associated with impaired kidney function was also observed 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 (Wei et al. 2012). 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).

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Table 2-5. Selected Epidemiological Studies of DEHP Exposure and Blood Pressure

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
James-Todd et al. 2016b, Cross-sectional (United States [NHANES])	965 cases of metabolic syndrome (464 men and 501 women) and 1,754 subjects without metabolic syndrome (924 men and 830 women), aged 20–80 years; participants in NHANES 2001–2010. Urine samples collected same day as blood pressure measurements.	Logistic regression adjusted for urinary creatinine, age, sex, race/ethnicity, total caloric intake, education, physical activity, smoking, and poverty	Prevalence OR for high blood pressure comparing highest quartile of urinary concentration with lowest (cases and controls grouped) Σ DEHP (MEHP, MEHHP, MEOHP) 0.13 (0.12, 0.15) (GM [95% CI]) Without metabolic syndrome: 0.12 (0.10, 0.13)	All: OR 1.56 (1.14, 2.12)* Men: OR 1.85 (1.12, 3.05)* Women: OR 1.24 (0.82, 1.88)
Lin et al. 2016, Cross-sectional (Taiwan)	794 adult students (243 men and 550 women; mean age 21.28 years), including 303 with and 486 without elevated blood pressure in childhood, from the YOTA study (recruited 1992–2000 from schools). Urine sample collected same day as blood pressure measurements.	Linear regression adjusted for age, gender, and smoking status	Association between systolic blood pressure (mm Hg) and log-transformed Cr-adjusted urinary metabolite concentration MEHP 1.7–38.99 μ g/g Cr MEHHP 15.86–43.16 μ g/g Cr MEOHP 10.18–26.56 μ g/g Cr	β 0.225 (NR) β 0.144 (NR) β 0.136 (NR)

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Table 2-5. Selected Epidemiological Studies of DEHP Exposure and Blood Pressure

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Trasande and Attina 2015, Cross-sectional (United States [NHANES])	1,329 children (688 boys and 641 girls) aged 8–19 years, participants in NHANES 2009–2012. Urine sample collected same day as blood pressure measurement.	Linear or logistic regression adjusted for sex, caloric intake, physical activity, poverty-income ratio, serum cotinine, urinary creatinine, BMI category, race/ethnicity, and age category, and subsample weighting	OR for blood pressure >90 th percentile for age/height z-score/sex per log-unit increase in urinary metabolite concentration		
			ΣDEHP	0.077–0.313 μM	OR 1.31 (0.90, 1.91)
			MEHP	NR	OR 1.09 (0.79, 1.50)
			MEHHP	NR	OR 1.27 (0.89, 1.81)
			MEOHP	NR	OR 1.18 (0.83, 1.66)
			MECPP	NR	OR 1.47 (0.95, 2.27)
			Association between blood pressure z-score and log-transformed urinary metabolite concentration		
			ΣDEHP	0.077–0.313 μM	Systolic: β 0.10 (0.03, 0.18)* Diastolic: β 0.09 (0.04, 0.17)*
			MEHP	NR	Systolic: β 0.06 (-0.03, 0.12) Diastolic: β 0.04 (-0.02, 0.10)
			MEHHP	NR	Systolic: β 0.09 (0.02, 0.16)* Diastolic: β 0.09 (0.01, 0.16)*
			MEOHP	NR	Systolic: β 0.08 (0.01, 0.16)* Diastolic: β 0.09 (0.002, 0.16)*
			MECPP	NR	Systolic: β 0.11 (0.03, 0.19)* Diastolic: β 0.07 (-0.01, 0.16)

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Table 2-5. Selected Epidemiological Studies of DEHP Exposure and Blood Pressure

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Werner et al. 2015, Cohort	369 pregnant women aged ≥18 years; participants in the Health Outcomes and Measures of the Environment Study recruited between 2003 and 2006. Urine samples were collected at 16 and 26 weeks (on average) of gestation. Blood pressure data included two readings in early gestation (<20 weeks) and the two highest blood pressures after 20 weeks. Physician diagnoses of pregnancy-induced hypertensive diseases were recorded, including maternal hypertension (systolic ≥140 mm Hg and diastolic ≥90 mm Hg), preeclampsia, HELLP syndrome, or eclampsia.	Linear regression adjusted for maternal race, maternal age at delivery, household income, education, marital status, serum cotinine concentration, weeks of gestation at blood pressure measurement, parity, BMI at 16 weeks of gestation and previous use of blood pressure medications	Change in blood pressure at <20 weeks per 10-fold increase in log-transformed creatinine-adjusted urinary metabolite concentration at 16 weeks		
			ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	53–159 µg/g Cr (average concentration from 16 and 26 weeks of gestation)	Diastolic β -0.4 (-1.6, 0.8) Systolic β -0.2 (-2.0, 1.6)
			Change in blood pressure at ≥20 weeks per 10-fold increase in creatinine-adjusted urinary metabolite concentration at 16 or 26 weeks		
			ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	53–159 µg/g Cr (average concentration from 16 and 26 weeks of gestation)	Diastolic 16 weeks: β -0.8 (-2.2, 0.5) 26 weeks: β 0.3 (-1.3, 1.9) Average: β -0.6 (-2.4, 1.3) Systolic 16 weeks: β -1.0 (-3.0, 1.0) 26 weeks: β -0.8 (-3.1, 1.6) Average: β -1.6 (-4.3, 1.2)
			RR for pregnancy-induced hypertensive disorder per 10-fold increase in creatinine-adjusted urinary metabolite concentration at 16 or 26 weeks		
			ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	53–159 µg/g Cr (average concentration from 16 and 26 weeks of gestation)	16 weeks: RR 0.85 (0.46, 1.58) 26 weeks: RR 1.42 (0.64, 3.13) Average: RR 1.09 (0.45, 2.66)

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Table 2-5. Selected Epidemiological Studies of DEHP Exposure and Blood Pressure

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Shiue and Hristova 2014, Cross-sectional (United States [NHANES])	20,293 adults (10,081 male and 10,212 female) aged ≥20 years, participants in NHANES 2009–2012. Urine sample collected same day as blood pressure measurement.	Logistic regression adjusted for urine creatinine, age at examination, sex, ethnicity, BMI, and subsample weighting	OR for high blood pressure (systolic ≥140 mm Hg and diastolic ≥90 mm Hg) with change (not specified) in log-transformed urinary metabolite concentration		
			MEHP	Normal blood pressure: 4.15±16.49 High blood pressure: 3.36±6.62	OR 1.03 (0.82–1.30)
			MEHHP	Normal blood pressure: 27.75±155.35 High blood pressure: 25.03±50.74	OR 1.21 (1.01–1.46)*
			MEOHP	Normal blood pressure: 16.45±97.03 High blood pressure: 15.22±25.48	OR 1.21 (1.01–1.45)*
			MECPP	Normal blood pressure: 40.10±249.63 High blood pressure: 38.52±64.13	OR 1.29 (1.04–1.59)*
			Shiue (2014a, 2014b) 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.		

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Table 2-5. Selected Epidemiological Studies of DEHP Exposure and Blood Pressure

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Trasande et al. 2013b Cross-Sectional (United States [NHANES])	2,463 children (1,276 boys and 1,187 girls) aged 8–19 years; NHANES. Urine sample collected same day as blood pressure measurement.	Linear or logistic regression adjusted for urinary creatinine, BMI category, race/ethnicity, age category, caregiver education, poverty-income ratio, sex, serum cotinine, caloric intake, and television watching	OR for blood pressure >90 th percentile for age/height z-score/sex per log-unit increase in urinary metabolite concentration ΣDEHP 0.166–0.704 M (MEHP, MEHHP, MEOHP, MECPP)	OR 0.94 (0.82, 1.08)
			Association between blood pressure z-score and log-transformed urinary metabolite concentration ΣDEHP(M 0.166–0.704 M MEHP, MEHHP, MEOHP, MECPP)	Systolic: β 0.04 (0.001, 0.08)* Diastolic: β -0.005 (-0.04, 0.03)

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

ΣDEHP = sum DEHP metabolites; BMI = body mass index; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; HELLP = hemolysis, elevated liver enzymes, low platelet count; IQR = interquartile range; MECPP = 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; OR = odds ratio; YOTA = Young Taiwanese Cohort

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In 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; Hazelton Washington 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). 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).

A potential effect on human heart muscle contractility was identified in *in vitro* studies. MEHP displayed a dose-dependent negative inotropic effect that weakened human atrial trabecular contractions at concentrations of 15–200 µg/mL, with an IC₅₀ of 85 µg/mL (Barry et al. 1990). 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. Evidence from animal studies suggests that altered blood pressure is secondary to renal toxicity following exposure to DEHP. Available animal data do not indicate that the cardiovascular system is a sensitive target of DEHP toxicity.

2.6 GASTROINTESTINAL

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

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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 (Shaffer et al. 1945). No other effects of exposure were noted.

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

In oral studies, pseudoductular lesions and altered acinar cell foci were observed in the pancreas of rats administered dietary DEHP at 3,000 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; Hazelton Washington 1992b; NTP 1982; Poon et al. 1997), or 7,899 mg/kg/day in mice (Hazelton Washington 1992a; NTP 1982; Toyosawa et al. 2001).

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 $\mu\text{g}/\text{m}^3$ DEHP) and 104 unexposed workers (average exposure, 0.26 $\mu\text{g}/\text{m}^3$ 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.

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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. 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; doses \leq 37.6 mg DEHP/kg/day were without hematological effect (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 \geq 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 at \leq 261.2 mg/kg/day (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 \geq 737 mg/kg/day, but not \leq 152 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). In mice, significantly reduced hemoglobin and hematocrit were observed in males and females exposed to dietary DEHP at doses \geq 1,209 and 2,888 mg/kg/day, respectively, for 28 days; no hematological changes were observed at dietary doses \leq 270 mg/kg/day (Myers 1992a). 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.

2.8 MUSCULOSKELETAL

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

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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). No adverse 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. No epidemiological data exist for DEHP exposure and the musculoskeletal endpoint. An adult monkey and multiple rodent studies indicate that the musculoskeletal system is not adversely affected from DEHP exposure.

2.9 HEPATIC

Overview. Human data on hepatic effects of DEHP are extremely limited. 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 $\mu\text{g}/\text{m}^3$ DEHP in the 3 factories) when compared with levels in 104 unexposed workers (average exposure, 0.26 $\mu\text{g}/\text{m}^3$ 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

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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 2-6. All five of these studies were cross-sectional in design. A positive association between hypertriglyceridemia and DEHP exposure was reported in a study of NHANES participants with and without metabolic syndrome (data from cases and non-cases were combined for regression analysis; James-Todd et al. 2016b), but other studies examining triglyceride levels observed no association (Lin et al. 2016; Trasande and Attina 2015, Trasande et al. 2013b; Yaghjian et al. 2015a, 2015b). Similarly, Lin et al. (2016) reported a negative relationship between MEHP in urine and high-density lipoprotein (HDL) cholesterol levels in 793 young adults in Taiwan, but no association was seen with other metabolites (MEHHP or MEOHP) or in other studies of this endpoint (James-Todd et al. 2016b; Trasande and Attina 2015, Trasande et al. 2013b; Yaghjian et al. 2015a, 2015b). None of the available studies indicated that DEHP urinary metabolite levels were associated with alterations in LDL or total cholesterol levels (Table 2-6).

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), 17 acute oral studies in rodents (Table 2-2) did not find exposure-related changes during microscopic examination of the liver following exposure to DEHP at 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).

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Table 2-6. Selected Epidemiological Studies of DEHP Exposure and Serum Lipid/Cholesterol Alterations

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)		Effect estimate (95% CI) ^a
James-Todd et al. 2016b, Case-control (United States)	965 cases of metabolic syndrome (464 men, 501 women) and 1,754 subjects without metabolic syndrome (924 men, 830 women), aged 20–80 years; participants in NHANES 2001–2010. Urine and blood samples collected same day.	Logistic regression adjusted for urinary creatinine, age, sex, race/ethnicity, total caloric intake, education, physical activity, smoking, and poverty	Prevalence OR for abnormal serum lipid and cholesterol levels comparing highest quartile of urinary concentration with lowest.		
			ΣDEHP (MEHP, MEHHP, MEOHP)	With metabolic syndrome: 0.13 (0.12, 0.15) (GM [95% CI]) Without metabolic syndrome: 0.12 (0.10, 0.13)	Hypertriglyceridemia OR 1.55 (1.12, 2.14)* Low HDL cholesterol OR 1.05 (0.74, 1.49)
Lin et al. 2016, Cross-sectional (Taiwan)	243 male and 550 female students (mean age 21.28 years), including 303 with and 486 without elevated blood pressure in childhood, from the YOTA study (recruited 1992–2000 from schools). Urine and blood samples collected same day.	Linear regression adjusted for age, gender, and smoking status	Association between serum lipid and cholesterol levels and log-transformed Cr-adjusted urinary metabolite concentration		
			MEHP	1.7–38.99 µg/g Cr	Log-triglycerides β 0.000 (NR) HDL cholesterol β -0.325* (NR) LDL cholesterol β 0.455 (NR)
			MEHHP	15.86–43.16 µg/g Cr	Log-triglycerides β -0.0004 (NR) HDL cholesterol β 0.344 (NR) LDL cholesterol β -0.537 (NR)
			MEOHP	10.18–26.56 µg/g Cr	Log-triglycerides β -0.011 (NR) HDL cholesterol β 0.262 (NR) LDL cholesterol β -1.129 (NR)

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Table 2-6. Selected Epidemiological Studies of DEHP Exposure and Serum Lipid/Cholesterol Alterations

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Trasande and Attina 2015, Cross-Sectional (United States [NHANES])	1,329 children (688 boys and 641 girls) aged 6–19 years, participants in NHANES 2009–2012. Urine and blood samples collected same day.		OR for abnormal serum lipid or cholesterol levels per log-unit increase in urinary metabolite concentration ΣDEHP 0.077–0.313 μM (not specified)	Triglycerides ≥100 mg/dL OR 0.91 (0.69, 1.19) HDL <40 mg/dL OR 0.89 (0.58, 1.36)
Yaghjian et al. 2015a, 2015b, Cross-sectional (United States)	6,005 women ≥18 years of age (not pregnant and not diabetic); participants in NHANES 1999–2004. Urine and blood samples collected same day.	Ordered logistic regression adjusted for age, race, education, poverty, total calories, BMI, total fat, physical activity, menopausal status/hormone use, alcohol consumption, and smoking	OR for higher quartile of serum lipid or total or LDL cholesterol level (or lower HDL cholesterol) per quartile increase in Cr-adjusted urinary metabolite levels ΣDEHP 19.59–58.66 μg/g Cr MEHP 1.49–5.95 μg/g Cr MEHHP 9.86–31.09 μg/g Cr	Triglycerides OR 0.94 (0.81, 1.09) Total cholesterol OR 1.00 (0.90, 1.11) HDL cholesterol OR 1.05 (0.95, 1.15) LDL cholesterol OR 1.08 (0.92, 1.26) Triglycerides OR 0.91 (0.78, 1.05) Total cholesterol OR 1.00 (0.90, 1.11) HDL cholesterol OR 1.02 (0.91, 1.13) LDL cholesterol OR 0.98 (0.85, 1.14) Triglycerides OR 0.94 (0.81, 1.09) Total cholesterol OR 1.01 (0.90, 1.14) HDL cholesterol

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Table 2-6. Selected Epidemiological Studies of DEHP Exposure and Serum Lipid/Cholesterol Alterations

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
				OR 1.05 (0.96, 1.15) LDL cholesterol OR 1.10 (0.94, 1.29)
			MEOHP 6.83–19.84 µg/g Cr	Triglycerides OR 0.99 (0.84, 1.16) Total cholesterol OR 0.98 (0.88, 1.10) HDL cholesterol OR 1.03 (0.94, 1.12) LDL cholesterol OR 1.12 (0.96, 1.31)
			MECPP 17.16–49.78 µg/g Cr	Triglycerides OR 1.15 (0.88, 1.50) Total cholesterol OR 0.95 (0.80, 1.13) HDL cholesterol OR 1.06 (0.93, 1.21) LDL cholesterol OR 1.14 (0.95, 1.36)

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Table 2-6. Selected Epidemiological Studies of DEHP Exposure and Serum Lipid/Cholesterol Alterations

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Trasande et al. 2013b, Cross-sectional (United States [NHANES])	1,276 male and 1,187 females aged 6–19 years; participants in 2003–2008 NHANES. Urine and blood samples collected same day.	Logistic regression adjusted for urinary creatinine, BMI category, race/ethnicity, age category, caregiver education, poverty-income ratio, sex, serum cotinine, caloric intake, and television watching	OR for abnormal serum lipid or cholesterol levels per log-unit increase in urinary metabolite concentration ΣDEHP (MEHP, MEHHP, MEOHP, MECPP) 0.166–0.704 mol/L	Triglycerides ≥100 mg/dL OR 1.05 (0.90, 1.22) HDL <40 mg/dL OR 0.94 (0.82, 1.08)

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

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 = 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; OR = odds ratio; YOTA = Young Taiwanese Cohort

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A few intermediate-duration studies have reported exposure-related hepatic lesions other than hepatocellular hypertrophy in rats and mice 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 1984). Because this finding was limited to a single animal at a low dose only, it is likely a spontaneous 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 $\leq 1,093$ 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). In mice, moderate focal coagulative necrosis was observed in the livers of B6C3F1 mice after exposure to dietary doses $\geq 1,209$ mg/kg/day for 13 weeks, but not dietary 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, Kupffer cells, and hepatocyte pigmentation 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 and hepatocyte pigmentation 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

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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). Similarly, no biologically relevant changes in hepatic serum enzyme levels have been reported in rats following acute- or intermediate-duration oral exposure up to 1,858 mg/kg/day (Astill et al. 1986; Myers 1992b; Poon et al. 1997) or chronic-duration oral exposure up to 939 mg/kg/day (David et al. 2000a). In mice, 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 triglyceride levels were seen in rats exposed to DEHP at doses >100 mg/kg/day (Astill et al. 1986; Barber et al. 1987; CMA 1984; Poon et al. 1997; Reddy et al. 1976). 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).

Animal Studies—Elevated Liver Weight and Hypertrophy, Peroxisomal Proliferation, Enzyme Induction. These endpoints are associated with hepatomegaly in animals and may reflect adaptation of

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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 PPAR α , 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

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associated with these effects in adult, non-pregnant rats and mice were 50–60 and 180 mg/kg/day, respectively (Blystone et al. 2010; Mitchell et al. 1985; NTP 2005; Sasaki et al. 2003). One gestational/lactation exposure study reported increased maternal liver weight at 5 mg/kg/day (Pocar et al. 2012). Thirty 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 $\geq 1,000$ 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 1984; 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

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induction was significantly elevated at $\geq 1,881$ 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 1984; 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. 1943).

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

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stress; (8) inhibition of gap-junctional intracellular communication (GJIC); and (9) genotoxicity. The role of specific key events in rodent liver cancer is 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 PPAR α , PPAR δ , and PPAR γ . PPAR α is expressed at higher levels in mouse and rat liver compared to human liver. In the liver, PPAR α plays a role in fatty acid uptake and transport, ketogenesis, and lipogenesis. The hallmarks of PPAR α activation include: (1) an increase in the number and size of peroxisomes (i.e., peroxisome proliferation); (2) increased expression, protein, 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). PPAR α 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 PPAR α -null mice).

Induction of peroxisomal and microsomal enzymes mediated by PPAR α contributes to an increase in the formation of reactive oxygen species (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 (Turuneen 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

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(Roberts et al. 2007; Rusyn and Corton 2012), and suppression of apoptosis and increased 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 PPAR α -mediated peroxisome proliferation (McKee et al. 2000).

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

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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 $\mu\text{g}/\text{m}^3$ DEHP in the three factories), serum urea and creatinine levels did not differ from those in 104 unexposed workers (Wang et al. 2015). Among 52 Taiwanese children exposed to foods contaminated with DEHP (duration of time unknown), serum blood urea nitrogen (BUN) and creatinine levels 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 reported an association between higher levels of DEHP metabolites in urine and increasing urinary albumin/creatinine ratio (ACR; ~3-fold increase in DEHP metabolites was associated with 0.55 mg/g increase in ACR). Elevated ACR indicates elevated protein levels in the urine and is a biomarker for kidney disease. However, the odds of micro- or macroalbuminuria (ACR ≥ 30 mg/g) were not increased in children with higher levels of DEHP metabolites in urine (odds ratio [OR] per log unit increase in DEHP exposure 1.11, 95% confidence interval [CI] 0.78–1.57; Trasande et al. 2014). Tsai et al. (2016) reported higher urinary ACR (1.43 ± 1.0 mg/mmol in group with exposure estimated to be >0.05 mg/kg/day, compared with 0.47 ± 0.33 mg/mmol in unexposed group; $p=0.006$; p for trend with dose <0.0001) among Taiwanese children who had consumed DEHP-contaminated foods, as well as a higher prevalence of microalbuminuria (12.9%, 9/70 children) in those children with the highest intake of such foods, compared with unexposed children (0%, $n=18$ children). No other studies were located regarding renal effects in humans after inhalation or oral exposure to DEHP.

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).

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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 $\leq 1,040$ 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 normally occurring renal tubule pigmentation and 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). Rao et al. (1990) reported lipofuscin pigments in the tubular epithelium of rats exposed to 1,900 mg/kg/day for 108 weeks. The lesions in rats are consistent with spontaneous nephropathy commonly observed in aged rats, and suggest that treatment with DEHP might accelerate the onset of the lesion in younger rats. 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, up to 3,000 mg/kg/day for up to 13 weeks, or up to 1,440 mg/kg/day for 17 weeks (Astill et al. 1986; Barber et al. 1987; CMA 1984; Dalgaard et al. 2000; Gray et al. 1977; NTP 1982; Poon et al. 1997; Shaffer et al. 1945). However, one study reported increased cellular pigmentation in the proximal tubule epithelium of male and female rats at 1,724 and 1,857.6 mg/kg/day, respectively, after dietary exposure for 13 weeks (Myers 1992b).

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 $\geq 2,888$ mg/kg/day, respectively, for 28 days (Myers 1992a). These lesions were not observed in male or female mice exposed to doses up to 2,600 mg/kg/day for 4–13 weeks (Myers 1992a; NTP 1982). Tubular regeneration was also observed in male and female mice exposed to

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1,100 mg/kg/day (only dose tested) for 28 weeks; hydronephrosis was also observed in exposed females (Toyosawa et al. 2001). 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). 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; 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). No exposure-related changes were observed in clinical chemistry measures in mice 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- and chronic-duration rat studies at doses ≥ 113 mg/kg/day (Blystone et al. 2010; Carpenter et al. 1953; David et al. 2000a; Gray et al. 1977; Myers 1992b; NTP 2005; Poon et al. 1997; Schilling et al. 2001) and in acute-duration studies following exposure to 1,000 mg/kg/day (Dostal et al. 1987; Hellwig et al. 1997). However, kidney weight changes did not occur in other rat studies at acute-duration doses of 500–1,100 mg/kg/day (Astill et al. 1986; 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); however, no kidney weight changes occurred in mice exposed to intermediate-duration doses up to

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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 PPAR α activation, because both wild-type and PPAR α knockout (-/-) mice develop kidney lesions after intermediate-duration dietary exposure (Kamijo et al. 2007; Ward et al. 1998). In fact, Kamijo et al. (2007) proposed that PPAR α activation protects against DEHP-induced renal toxicity because PPAR α 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).

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).

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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).

Single doses of up to 19,800 mg/kg DEHP were applied to rabbit skin using a modified FDA cuff test procedure. There was no evidence of dermal irritation 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.

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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. Data regarding potential endocrine effects in animals following DEHP exposure were available from one inhalation study and numerous oral studies.

Epidemiology Studies—Thyroid Dysfunction. Effects of DEHP exposure on thyroid function (serum levels of triiodothyronine [T3], thyroxine [T4], and thyroid stimulating hormone [TSH]) have been evaluated in 10 epidemiological studies in which DEHP exposure was evaluated using urinary metabolite biomarkers (Table 2-7).

Five studies examining thyroid hormone levels in pregnant women did not provide 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 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 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).

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Table 2-7. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference and study type	Population	Method of analysis	Urinary metabolite levels	Regression coefficient (β) (95% CI) ^a	
Pregnant women					
Huang et al. 2018, Cohort/cross-sectional (Taiwan, China)	98 pregnant women referred for amniocentesis (2013–2014); mean age 35.0 years. Maternal blood and urine samples collected during each trimester (median 18, 26, and 39 weeks of gestation); cord blood collected at delivery.	Linear mixed models repeated measures analysis adjusted for maternal age at enrollment, gestational age at sample collection, urinary creatinine, and serum TBG levels For cord blood analysis, models were adjusted for maternal age at enrollment, urinary creatinine, and maternal thyroid level	Change in ln-transformed maternal serum thyroid hormone per ln-unit increase in ln-transformed urinary metabolite		
			Σ DEHP	Visit 1: 21.64 (16.44, 28.25) ng/mL; Visit 2: 30.68 (24.51, 38.39) ng/mL; Visit 3: 39.34 (31.60, 48.97) ng/mL (GM [95%CI])	TSH: -0.074 (-0.161, 0.013) TT3: -0.022 (-0.046, 0.003) TT4: 0.003 (-0.015, 0.021) FT4: 0.007 (-0.017, 0.030)
			MEHP	Visit 1: 2.43 (1.67, 3.52) ng/mL; Visit 2: 3.45 (2.43, 4.91) ng/mL; Visit 3: 2.49 (1.60, 3.87) ng/mL	TSH: -0.006 (-0.059, 0.047) TT3: -0.0001 (-0.016, 0.015) TT4: -0.002 (-0.013, 0.009) FT4: 0.006 (-0.008, 0.020)
			MEHHP	Visit 1: 2.67 (1.75, 4.08) ng/mL; Visit 2: 5.33 (3.63, 7.82) ng/mL; Visit 3: 9.69 (7.27, 12.91) ng/mL	TSH: -0.018 (-0.072, 0.037) TT3: -0.013 (-0.028, 0.002) TT4: -0.005 (-0.016, 0.007) FT4: -0.008 (-0.023, 0.006)
			MEOHP	Visit 1: 3.41 (2.45, 4.75) ng/mL; Visit 2: 5.36 (4.06, 7.08) ng/mL; Visit 3: 8.38 (6.68, 10.52) ng/mL	TSH: -0.083 (-0.157, -0.009)* TT3: -0.012 (-0.033, 0.010) TT4: 0.001 (-0.015, 0.016) FT4: -0.011 (-0.031, 0.010)
			MECCP	Visit 1: 6.15 (4.37, 8.65) ng/mL; Visit 2: 9.89 (7.95, 12.30) ng/mL; Visit 3: 12.46 (10.03, 15.50) ng/mL	TSH: -0.051 (-0.124, 0.021) TT3: -0.027 (-0.047, -0.006)* TT4: 0.004 (-0.011, 0.019) FT4: -0.008 (-0.027, 0.011)
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.					

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Table 2-7. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference and study type	Population	Method of analysis	Urinary metabolite levels	Regression coefficient (β) (95% CI) ^a	
Johns et al. 2016, Case-control (United States [Massachusetts])	439 pregnant women (116 cases of preterm birth and 323 term birth controls); nested case-control study of women from LifeCodes prospective birth cohort.	Linear mixed model adjusted for urinary SG, gestational age at time of sample collection, maternal age at enrollment, BMI at time of sample collection, and health insurance provider	Percent change in serum thyroid hormone per interquartile increase in urinary metabolite		
			Σ DEHP	Visit 1: 0.39 (3.16) μ mol/L; Visit 2: 0.38 (3.01); Visit 3: 0.32 (3.04); Visit 4: 0.42 (3.18) (GM [GSD]); SG-adj)	TT3: 0.82 (-0.77, 2.41) FT4: 4.09 (-1.12, 9.29) TT4: 0.87 (-0.17, 1.91) TSH: -4.33 (-9.23, 0.84)
			MEHP	Visit 1: 10.6 (3.52); Visit 2: 10.9 (3.39); Visit 3: 9.46 (3.28); Visit 4: 9.83 (3.52)	TT3: 0.28 (-1.29, 1.85) FT4: 4.15 (-0.87, 9.16) TT4: 1.29 (0.26, 2.32)* TSH: -5.31 (-10.1, -0.23)*
			MEHHP	Visit 1: 34.7 (3.37); Visit 2: 34.8 (3.10); Visit 3: 27.2 (3.21); Visit 4: 9.83 (3.33)	TT3: 0.97 (-0.55, 2.5) FT4: 2.67 (-2.27, 7.62) TT4: 0.66 (-0.34, 1.66) TSH: -3.95 (-8.67, 1.01)
			MEOHP	Visit 1: 18.6 (3.28); Visit 2: 18.3 (3.03); Visit 3: 15.6 (3.19); Visit 4: 20.9 (3.22)	TT3: 1.08 (-0.41, 2.58) FT4: 3.89 (-0.99, 8.77) TT4: 0.86 (-0.13, 1.84) TSH: -3.74 (-8.38, 1.15)
			MECPP	Visit 1: 44.4 (3.35); Visit 2: 42.6 (3.25); Visit 3: 36.8 (3.31); Visit 4: 49.3 (3.35)	TT3: 0.86 (-0.83, 2.54) FT4: 4.89 (-0.52, 10.3) TT4: 0.86 (-0.25, 1.97) TSH: -3.98 (-9.17, 1.51)
Repeated measures analysis with cases and controls combined.					

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Table 2-7. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference and study type	Population	Method of analysis	Urinary metabolite levels	Regression coefficient (β) (95% CI) ^a
Yao et al. 2016, Cohort/Cross-sectional (China)	2,521 pregnant women, mean age 26years, members of Ma'anshan Birth cohort, recruited at 1 st prenatal visit (<14 weeks of gestation) at the Maternal and Child Health Center in Ma'anshan city.	Linear regression models adjusted for maternal age, prepregnancy BMI, parity, race, education level, residence in the previous 6 months, cigarette smoking, alcohol consumption, and gestational week at blood withdrawal	Mean change in maternal serum thyroid hormone per 1-SD increase in ln-transformed urinary metabolite	
			MEHP NR	InTSH: 0.101 (0.055, 0.147)* TT4: -0.163 (-0.261, -0.065)* FT4: -0.013 (-0.020, -0.006)* TT3: -0.453 (-1.771, 0.864)
			MEHHP NR	InTSH: 0.132 (0.086, 0.177)* TT4: -0.173 (-0.270, -0.075)* FT4: -0.011 (-0.017, -0.004)* TT3: 0.993 (-0.321, 2.306)
			MEOHP NR	InTSH: 0.051 (0.005, 0.097) TT4: -0.033 (-0.0131, 0.065) FT4: -0.002 (-0.009, 0.004) TT3: 0.509 (-0.806, 1.824)
			Maternal serum and urine samples collected at 1 st prenatal visit (mean 10 weeks of gestation); cord serum collected at delivery.	Cord serum models additionally adjusted for infant sex, gestation age at delivery, and delivery mode
Johns et al. 2015, Cohort/cross-sectional (Puerto Rico)	106 pregnant women aged 18–40 years, members of PROTECT birth cohort, recruited at 14 weeks of gestation from prenatal clinics and hospitals.	Linear regression models adjusted for age at enrollment, prepregnancy BMI, and urinary SG	Percent change in serum thyroid hormone per interquartile increase in urinary metabolite	
			Σ DEHP NR	FT3: 0.05 (-4.49, 4.49) FT4: -8.02 (-15.3, -0.8)* TSH: 2.79 (-10.8, 18.6)
			MEHP Visit 1: 1.61–6.36; Visit 3: 1.69–6.73 (SG-adj)	NR
			MEHHP Visit 1: 6.14–19.9; Visit 3: 7.28–16.9	NR
Urine and serum samples collected at		MEOHP Visit 1: 5.57–16.5; Visit 3: 6.22–14.8	NR	

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Table 2-7. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference and study type	Population	Method of analysis	Urinary metabolite levels	Regression coefficient (β) (95% CI) ^a
	1 st and 3 rd prenatal visits (16–20 and 24–28 weeks of gestation).		MECPP Visit 1: 12.7–31.4; Visit 3: 13.4–29.3	NR
			Cross-sectional analysis (same day serum and urine samples) using visit 3 data only; no significant association seen with visit 1 data only or in longitudinal analysis.	
Huang et al. 2007, Cross-sectional (Taiwan, China)	76 pregnant women referred for amniocentesis (2005–2006) due to abnormal α -fetoprotein or free β -hCG or advanced age; mean age 33.6 years; mean week of gestation=27.9.	Multiple linear regression adjusted for age, BMI, gestational age, and other phthalate monoesters	Change in serum thyroid hormone per log-unit increase in urinary metabolite MEHP 31.4–121.0 $\mu\text{g/g Cr}$	FT4: -0.015 (NR) TT4: = -0.007 (NR)
	Urine and blood samples collected on same day at referral.		One outlier excluded due to hypothyroidism.	
Other populations				
Kuo et al. 2015, Cohort (Taiwan)	148 mother-child pairs recruited from hospital between 2009 and 2010.	Multiple linear regression adjusted for age, infant gender, prepregnancy BMI, weight gain, gestational age, parity, educational level, cigarette smoking, alcohol intake, maternal serum collected at delivery. TSH, and other urinary phthalate monoesters	Association between log-transformed cord blood TSH and maternal urinary metabolite level MEHP 8.19–19.34 $\mu\text{g/g Cr}$ MEHHP 14.84–33.81 $\mu\text{g/g Cr}$ MEOHP 14.68–31.59 $\mu\text{g/g Cr}$	TSH: -1.342 (NR) TSH: -2.375 (NR) TSH: 2.676 (NR)
	Single maternal urine sample collected during 3 rd trimester; cord blood collected at delivery.			

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Table 2-7. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference and study type	Population	Method of analysis	Urinary metabolite levels	Regression coefficient (β) (95% CI) ^a	
Dirtu et al. 2013, Case-control (Belgium)	152 obese individuals recruited at the entry of a 12-month weight-loss program between November 2009 and February 2012 (46 men, 106 women; aged 18–84 years) and 43 non-obese, age- and sex-matched controls (12 men, 30 women; aged 19–59 years). Urine and blood samples collected at baseline (obese and control) and during weight-loss treatment of obese individuals.	Linear regression adjusted for age and gender	Association between serum thyroid hormone level and urinary phthalate metabolite concentration in non-obese controls (male and female)		
			Σ DEHP	Controls: 27–53	FT4: 0.12 TSH: 0.38*
			MEHP	Controls: 2–5	FT4: 0.08 TSH: 0.27
			MEHHP	Controls: 9–19	FT4: 0.12 TSH: 0.31
			MEOHP	Controls: 3–9	FT4: 0.10 TSH: 0.40*
			MECPP	Controls: 12–20	FT4: 0.10 TSH: 0.38*
			Association between serum thyroid hormone level and urinary phthalate metabolite concentration (at baseline) in obese cases		
			Σ DEHP	Cases: 30–61	FT4: -0.02 TSH: 0.04
			MEHP	Cases: 2–5	FT4: 0.00 TSH: -0.05
			MEHHP	Cases: 10–25	FT4: 0.00 TSH: 0.01
			MEOHP	Cases: 4–11	FT4: -0.03 TSH: 0.04
			MECPP	Cases: 12–22	FT4: -0.07 TSH: 0.03

95% CI values were not reported; $p > 0.1$ for all. Gender-specific results also did not show any significant associations for DEHP metabolites.

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Table 2-7. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference and study type	Population	Method of analysis	Urinary metabolite levels	Regression coefficient (β) (95% CI) ^a
Meeker et al. 2011, Cross-sectional (United States)	1,346 adults (≥ 20 years of age) and 329 adolescents (ages 12–19) from 2007–2008 NHANES participants. Urine and blood samples collected on same day.	Multivariable linear regression adjusted for age, race, BMI, In-serum cotinine, In-urinary creatinine, and In-urinary iodine	Change in serum thyroid hormone level per ln-unit increase in urinary metabolite in adolescents	
			MEHP <LOD–4.5 $\mu\text{g/g Cr}$	FT3: 0.0081 (0.00035, 0.016) TT3: 4.00 (1.97, 6.03)* FT4: -0.0060 (-0.025, 0.013) TT4: 0.11 (-0.1, 0.33) TSH: -0.004 (-0.055, 0.046)
			MEHHP 10.3–45.32 $\mu\text{g/g Cr}$	FT3: 0.0084 (-0.0026, 0.019) TT3: 3.85 (1.44, 6.27)* FT4: 0.0003 (-0.017, 0.018) TT4: 0.089 (-0.11, 0.29) TSH: 0.045 (-0.026, 0.12)
			MEOHP 5.79–24.74 $\mu\text{g/g Cr}$	FT3: 0.0082 (-0.0036, 0.020) TT3: 4.24 (1.72, 6.75)* FT4: -0.0007 (-0.018, 0.017) TT4: 0.094 (-0.099, 0.29) TSH: 0.048 (-0.028, 0.12)
			MECPP 16.7–64.8 $\mu\text{g/g Cr}$	FT3: 0.011 (-0.00096, 0.023) TT3: 4.70 (1.97, 7.43)* FT4: 0.0033 (-0.018, 0.024) TT4: 0.15 (-0.046, 0.35) TSH: 0.048 (-0.029, 0.13)
			Change in thyroid hormone level per ln-unit increase in urinary metabolite in adults.	
			MEHP <LOD–5.20 $\mu\text{g/g Cr}$	FT3: -0.00003 (-0.0085, 0.0085) TT3: -0.83 (-2.17, 0.051) FT4: -0.0054 (-0.020, 0.0091) TT4: -0.13 (-0.22, -0.045)* TSH: 0.028 (-0.0013, 0.057)

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Table 2-7. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference and study type	Population	Method of analysis	Urinary metabolite levels	Regression coefficient (β) (95% CI) ^a
			MEHHP 9.84–37.0 $\mu\text{g/g Cr}$	FT3: -0.0021 (-0.0082, 0.0040) TT3: -1.49 (-2.72, -0.26)* FT4: -0.010 (-0.027, 0.0066) TT4: -0.21 (-0.32, -0.11)* TSH: 0.046 (0.012, 0.081)*
			MEOHP 5.43–20.5 $\mu\text{g/g Cr}$	FT3: -0.0024 (-0.0082, 0.0035) TT3: -1.36 (-2.54, -0.18)* FT4: -0.0088 (-0.026, 0.0081) TT4: -0.19 (-0.36, -0.086)* TSH: 0.047 (0.012, 0.082)*
			MECPP 15.4–50.8 $\mu\text{g/g Cr}$	FT3: 0.0004 (-0.0063, 0.0070) TT3: -1.37 (-2.89, 0.15) FT4: -0.011 (-0.027, 0.0057) TT4: -0.23 (-0.33, -0.13)* TSH: 0.041 (0.006, 0.077)*
Analyses weighted for sampling strategy.				
Boas et al. 2010, Cross-sectional (Denmark)	758 children aged 4–9 years, members of birth cohort who agreed to provide spot urine and blood samples.	Multivariate linear regression adjusted for sex and age	Change in serum thyroid hormone per log-unit increase in Cr-corrected urinary metabolite concentration	
			Σ DEHP NR	FT3: 0.04 (-0.12, 0.21) TT3: 0.06 (-0.02, 0.14) FT4: 0.05 (-0.4, 0.49) TT4: 1.91 (-2.64, 6.46) TSH: 0.04 (-0.01, 0.08)
	Urine and blood samples collected during clinical examination.		MEHP 4.1–11 $\mu\text{g/g Cr}$ (male); 4.1–12 $\mu\text{g/g Cr}$ (female)	FT3: 0.04 (-0.09, 0.18) TT3: 0.02 (-0.05, 0.08) FT4: -0.06 (-0.42, 0.3) TT4: -0.65 (-4.37, 3.07) TSH: 0.03 (-0.01, 0.07)

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Table 2-7. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference and study type	Population	Method of analysis	Urinary metabolite levels	Regression coefficient (β) (95% CI) ^a
			MEHHP 33–84 $\mu\text{g/g}$ Cr (male); 36–81 $\mu\text{g/g}$ Cr (female)	FT3: 0.05 (-0.11, 0.2) TT3: 0.05 (-0.02, 0.13) FT4: 0.15 (-0.25, 0.55) TT4: 2.52 (-1.6, 6.64) TSH: 0.03 (-0.01, 0.08)
			MEOHP 17–42 $\mu\text{g/g}$ Cr (male); 18–41 $\mu\text{g/g}$ Cr (female)	FT3: 0.04 (-0.12, 0.2) TT3: 0.05 (-0.02, 0.13) FT4: 0.05 (-0.38, 0.47) TT4: 1.86 (-2.53, 6.24) TSH: 0.04 (0.00, 0.09)
			MECPP 29–68 $\mu\text{g/g}$ Cr (male); 33–75 $\mu\text{g/g}$ Cr (female)	FT3: 0.01 (-0.15, 0.17) TT3: 0.05 (-0.03, 0.13) FT4: -0.08 (-0.52, 0.36) TT4: 0.77 (-3.75, 5.29) TSH: 0.03 (-0.02, 0.07)
Cr-corrected analysis for all children (girls and boys combined); $p > 0.05$ for all.				

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Table 2-7. Summary of Epidemiological Studies of DEHP Exposure and Thyroid Hormone Levels

Reference and study type	Population	Method of analysis	Urinary metabolite levels	Regression coefficient (β) (95% CI) ^a
Meeker et al. 2007, Cross-sectional (United States [Massachusetts])	408 male partners of subfertile couples, ages 18–55 years old, evaluated at fertility clinic between January 2000 and May 2004.	Multivariate linear regression adjusted for age, BMI, current smoking, and time of day of blood sample. TSH concentrations log-transformed; FT3 and testosterone untransformed	Change in serum thyroid hormone per interquartile range increase in ln-transformed, SG-adjusted urinary metabolites	
			MEHP 3.16–21.3	FT4: -0.013 (-0.042, 0.017) TT3: -0.021 (-0.042, -0.001)* TSH: 0.97 (0.9, 1.04)
			MEHHP 23.4–113	FT4: 0.008 (-0.017, 0.033) TT3: -0.002 (-0.03, 0.025) TSH: 0.98 (0.88, 1.08)
	Urine and blood samples collected on same day.		MEOHP 16.3–71.3	FT4: 0.013 (-0.01, 0.035) TT3: 0.003 (-0.024, 0.028) TSH: 0.97 (0.88, 1.06)

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

Σ DEHP = sum DEHP metabolites; BMI = body mass index; CI = confidence interval; DEHP = di(2-ethylhexyl)phthalate; FT3 = free triiodothyronine; FT4 = free thyroxine; GM = geometric mean; GSD = geometric standard deviation; hCG = human chorionic gonadotropin; 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; PROTECT = Puerto Rico Test Site for Exploring Contamination Threats; SG = specific gravity; SG-adj = specific gravity adjusted; TBG = thyroxine-binding globulin; TSH = thyroid-stimulating hormone; TT3 = total triiodothyronine; TT4 = total thyroxine

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In two studies, no associations were observed between maternal urinary DEHP metabolite levels and cord serum thyroid hormone levels (Huang et al. 2018; Yao et al. 2016). In cross-sectional studies of other populations, associations were seen between DEHP urinary metabolite levels and increased serum TSH in adults (Dirtu et al. 2013; Meeker et al. 2011) but not adolescents (Meeker et al. 2011) or in obese individuals (Dirtu et al. 2013); decreased total T3 and T4 in adults (Meeker et al. 2007, 2011); and increased total T3 in adolescents (Meeker et al. 2011). No associations between DEHP metabolites in urine and serum thyroid hormone levels were observed in a cross-sectional study of children 4–9 years old (Boas et al. 2010).

Animal Studies—Thyroid/Parathyroid Gland. Only one animal study was found in the literature that evaluated the function of the thyroid gland. In this study, there were no changes in serum thyroid hormones in PND 21 or 63 offspring born to Sprague-Dawley rat dams exposed to DEHP at doses up to 400 mg/kg/day from GD 6 to PND 20 (Kobayashi et al. 2006).

No changes in thyroid/parathyroid weight or histology were observed in any 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 (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. DEHP was shown to be a thyroid receptor antagonist, and inhibited the binding of T3 to the purified thyroid receptor.

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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 3,000 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; Hazelton Washington 1992b; NTP 1982; Poon et al. 1997), or 7,899 mg/kg/day in mice (Hazelton Washington 1992a; NTP 1982; Toyosawa et al. 2001).

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 $\geq 1,724$ 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

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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) or chronic-duration studies at doses up to 1,821 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).

In contrast, *increased* relative adrenal 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

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reproductive study (Blystone et al. 2010; NTP 2005). Adrenal weight changes were not observed in parental females. 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 (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 PPAR α 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.

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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). 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. There is no evidence of thyroid damage following DEHP exposure from the single available animal study that specifically evaluated thyroid function. In animals, there is some evidence for adverse effects in the adrenal and pituitary glands. Animal data

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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. 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 2-8. In studies that examined the risk for asthma symptoms or wheezing (Gascon et al. 2015a; Ku et al. 2015; Whyatt et al. 2014), 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. No association was seen in the other studies, possibly due to bias or analysis limited to a subset of DEHP metabolites (Ku et al. 2015; Whyatt et al. 2014).

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 (β 0.50 and 0.36, respectively) associated with higher serum IgE in children 8 years of age (Ku et al. 2015). A cross-sectional study of children 3–5 years of age did not find an association between the children's DEHP metabolite levels and IgE sensitization (Bekö et al. 2015), although further confirmation of this result is needed. 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.

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). Prenatal DEHP exposure does not appear to be associated with atopic dermatitis or eczema in early childhood, based on the findings of three birth cohort studies in Poland, Spain, and New York (Gascon et

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Table 2-8. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Ashley-Martin et al. 2015, Cohort (Canada)	1,137 children, members of birth cohort (Maternal-Infant Research on Environmental Chemicals); pregnant women (14 weeks of gestation) recruited from 10 cities between 2008 and 2011. Single urine sample collected during first trimester. Cord blood samples collected at birth.	Bayesian hierarchical logistic regression adjusted for maternal age and SG	OR for elevated IgE ≥ 0.5 ku/L in cord blood with log-transformed maternal urinary metabolite concentration		
			Σ DEHP	NR	OR 1.0 (0.7–1.5)
			MEHP	IgE ≥ 0.5 ku/L: 2.6 (2.7) IgE < 0.5 ku/L: 2.6 (2.5) (GM [GSD])	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
			OR for elevated IL-33 and TSLP (both $\geq 80^{\text{th}}$ percentile; pg/mL) in cord blood with log-transformed maternal urinary metabolite concentration		
			Σ DEHP	NR	OR 1.0 (0.7–1.3)
			MEHP	IL-33 and TSLP $\geq 80^{\text{th}}$ percentile: 2.5 (2.6) IL-33 and TSLP $< 80^{\text{th}}$ percentile: 2.7 (2.5) (GM [GSD])	NR
			MEHHP	IL-33 and TSLP $\geq 80^{\text{th}}$ percentile: 9.4 (2.6) IL-33 and TSLP $< 80^{\text{th}}$ percentile: 10.7 (2.5)	NR
			MEOHP	IL-33 and TSLP $\geq 80^{\text{th}}$ percentile: 6.8 (2.5) IL-33 and TSLP $< 80^{\text{th}}$ percentile: 7.5 (2.3)	NR

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Table 2-8. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Bekö et al. 2015, Case-control (Denmark)	200 cases, children 3–5 years old with at least two parentally-reported conditions (asthma, allergic rhinoconjunctivitis, or eczema), from the city of Odense or its suburbs, and 300 randomly selected controls. Blood and urine samples collected on the day of clinical examination.	Logistic regression adjusted for breast-feeding, allergic predisposition, sex, social class, renovation due to moisture damage and cat and dog allergens in the dust	OR for IgE sensitization with log-transformed urinary metabolite concentration		
			MEHP	Cases: IgE-: 3.7; IgE+: 4.01 (median) Controls: 5.18	NR (NS)
			MEHHP	Cases: IgE-: 31.7; IgE+: 33.2 Controls: 33.5	NR (NS)
			MEOHP	Cases: IgE-: 13.3; IgE+: 16.0 Controls: 17.5	NR (NS)
			MECPP	Cases: IgE-: 29.9; IgE+: 31.5 Controls: 36.6	Among asthma patients: OR 2.9 (1.12, 7.6)*
ORs for IgE sensitization among controls and among cases with rhinoconjunctivitis and atopic dermatitis were not significant (not reported)					
Ku et al. 2015, Cohort (Taiwan)	171 children, members of birth cohort; pregnant women recruited during the 3 rd trimester between December 2000 and November 2001. Single maternal urine sample collected at enrollment; children's urine samples collected at ages 2, 5, and 8. At 8 years of age, children were evaluated for asthma symptoms and blood sample collected for IgE measurement.	Logistic regression adjusted for parental allergies and family members' smoking status (wheezing, asthma) and linear regression, adjusted for sex and parental allergies	OR for wheezing or asthma comparing highest quintile (>80 th percentile) of maternal urinary metabolite concentration with those <80 th percentile		
			ΣDEHP (MEHP, MEHHP)	50.22 (42.22, 59.72) µg/g Cr (GM [95% CI])	Wheezing OR 3.12 (0.98–9.98) Asthma OR 0.81 (0.21–3.14)
			MEHP	16.90 (14.49, 19.72) µg/g Cr	NR
			Association between log-transformed serum IgE and log-transformed metabolite concentration in maternal urine		
			ΣDEHP (MEHP, MEHHP)	50.22 (42.22, 59.72) µg/g Cr (GM [95% CI])	Allergic children β 0.20 (NR) Non-allergic children β 0.12 (NR) All children β 0.03 (NR)

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Table 2-8. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
			MEHP 16.90 (14.49, 19.72) µg/g Cr	Allergic children β 0.50* (NR) Non-allergic children β 0.24 (NR) All children β 0.38 (NR)
Association between log-transformed serum IgE and log-transformed metabolite concentration in child's urine at 5 years of age				
			ΣDEHP NR (MEHP, MEHHP)	Allergic children β 0.29 (NR) Non-allergic children β -0.14 (NR) All children β 0.14 (NR)
			MEHP 11.9 µg/g Cr (GM)	Allergic children β 0.36* (NR) Non-allergic children β -0.22 (NR) All children β 0.10 (NR)
No significant association between serum IgE and metabolite concentration in child's urine at 2 years of age				

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Table 2-8. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Stelmach et al. 2015, Cohort (Poland)	147 children (82 boys, 65 girls), members of birth cohort (Polish Mother and Child Cohort); pregnant women recruited during first trimester from maternity units or clinics. Single maternal urine sample collected during 3 rd trimester. Children's allergy symptoms determined at age 2 years by parental report supplemented by medical chart review.	Logistic regression adjusted for atopy in family, father's education, frequency of cleaning, and breastfeeding (atopic dermatitis) or pets at home during pregnancy and breastfeeding (food allergy)	OR for atopic dermatitis or food allergy with log-transformed maternal urinary metabolite concentration		
			ΣDEHP	1.73–37.75 µg/g Cr	Atopic dermatitis OR 0.36 (0.08, 1.51) Food allergy OR 0.97 (0.29, 3.30)
			MEHP	0.04–0.64 µg/g Cr	Atopic dermatitis OR 0.47 (0.13, 1.75) Food allergy OR 0.45 (0.13, 1.58)
			MEHHP	0.11–20.57 µg/g Cr	Atopic dermatitis OR 0.70 (0.29, 1.70) Food allergy OR 1.02 (0.45, 2.31)
			MEOHP	0.69–6.54 µg/g Cr	Atopic dermatitis OR 0.46 (0.14, 1.59) Food allergy OR 1.02 (0.34, 3.04)
There were no significant associations between atopic dermatitis or food allergy and children's urinary metabolite concentrations					

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Table 2-8. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Gascon et al. 2015a, Cohort (Spain)	391 children (238 boys and 224 girls), members of birth cohort (INMA/ Environment and Childhood); pregnant women recruited during first trimester at public hospital or health center. Urine samples collected during 1 st and 3 rd trimesters and results averaged for analysis. Allergy outcomes in the prior 6 or 12 months determined by maternal questionnaire at 6 and 14 months and 4 and 7 years of age. Atopy determined as IgE \geq 2 kU/L to common allergens measured in blood sample collected at age 4 years.	Logistic regression, adjusted for maternal education, number of siblings, maternal smoking during pregnancy, maternal history of asthma/allergy, and maternal BMI	RR for allergic and respiratory outcomes between birth and age 7, with doubling of log ₂ -transformed maternal urinary metabolite concentration	
			Σ DEHP 69.5–147.9 μ g/g Cr (MEHP, MEHHP, MEOHP, MECPP)	Wheeze RR 1.25 (1.04–1.50)* Eczema RR 1.00 (0.83–1.20)
			RR for asthma at age 7 or atopy at age 4, with doubling of log ₂ -transformed maternal urinary metabolite concentration	
			Σ DEHP 69.5–147.9 μ g/g Cr	Asthma at age 7 RR 1.38 (1.05–1.82)* Atopy at age 4 RR 1.13 (0.60–2.11)
			MEHP 7.3–17.2 μ g/g Cr	NR
			MEHHP 17.9–41.5 μ g/g Cr	NR
Wang et al. 2014, Cohort/cross-sectional (Taiwan)	483 children (244 boys and 239 girls), members of birth cohort (Taiwan Birth Panel cohort); pregnant women recruited during 3 rd trimester from selected hospitals. Single maternal urine sample collected during 3 rd trimester; children's urine samples collected at ages 2 and 5 years. Atopic disorders determined by parental questionnaire at ages 2 and 5 years. Children's blood samples collected at ages 2 and 5 for serum IgE determination.	Linear regression adjusted for gestational age, maternal education, maternal history of atopy, and prenatal environmental tobacco smoke exposure (IgE), or logistic regression, adjusted for gender,	Association between log-transformed serum IgE at age 2 years and log-transformed child's urinary metabolite concentration at age 2 years	
			MEHP 16.01 (1.12) μ g/g Cr (GM [SE])	All children: β 0.191* (NR) Boys: β 0.256* (NR) Girls: β 0.107 (NR)
			OR for atopic dermatitis comparing highest quartile of child's urinary metabolite concentration at age 2 years with lowest quartile	
			MEHP 16.01 (1.12) μ g/g Cr (GM [SE])	Atopic dermatitis at age 2 years OR 1.31 (0.50, 3.45) Atopic dermatitis at age 5 years OR 1.76 (0.67, 4.64)

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Table 2-8. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
		gestational age, maternal education, maternal history of atopy, and pre-natal ETS exposure		
Whyatt et al. 2014, Cohort (United States [New York])	300 children (137 boys and 163 girls), members of birth cohort (CCCEH); black or Dominican mothers recruited prior to 20 th week of pregnancy, between 1998 and 2004. Single maternal urine sample collected during 3 rd trimester (mean 34 weeks of gestation); children's urine samples collected at ages 3, 5, and 7 years. Asthma determined by questionnaire administered to parent when children were ages 5, 6, 7, 9, and 11 years of age	Poisson regression with robust standard error estimation using generalized estimating equations, adjusted for maternal asthma, household smoke exposure, maternal prenatal BPA exposure, maternal prenatal demoralization ^b , maternal prenatal urinary SG, and child age	RR for asthma symptoms, compared with nonasthmatics, comparing highest tertile of ln-transformed maternal urinary metabolite concentration with lowest MEHHP 10.6–50.0	History of asthma symptoms RR 0.97 (0.74, 1.28) Diagnosis of current asthma RR 1.03 (0.89, 1.20) History of asthma but diagnosis of not current asthma RR 0.95 (0.82, 1.10)

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Table 2-8. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Bertelsen et al. 2013, Cross-sectional (Norway)	623 children, members of birth cohort (Environment and Childhood Asthma cohort); healthy infants born in Oslo were enrolled between January 1, 1992 and March 31, 1993. A subset of the cohort with lung function measurement at birth and/or participants in 2-year followup study were invited to 10-year followup; of those who agreed, 623 were selected, with preferential sampling of those with asthma (21% prevalence). Current asthma defined by history of asthma and symptoms, asthma medicine use, or clinical signs in the previous 12 months. Urine samples collected same day as clinical examination.	Logistic regression adjusted for urine SG, sex, parental asthma, and household income	OR for current asthma with log-transformed urinary metabolite concentration	Highest versus lowest quartile: OR 1.6 (0.83, 3.2) Per log-IQR change: OR 0.99 (0.75, 1.3)	
			ΣDEHP		0.58–1.18 μmol/L (SG-adj)
			MEHP		5.0–12.5
			MEHHP		56.9–116.4
			MEOHP		36.1–75.3
			MECPP	71.7–153.2	NR
Hoppin et al. 2013, Cross-Sectional (United States [NHANES])	2,325 children ≥6 years old; participants in NHANES 2005–2006 with complete data. Allergic symptoms determined by questionnaire; urine samples collected same day.	Logistic regression adjusted for age, race, gender, BMI, creatinine, and cotinine	OR for current allergic symptoms per log-unit increase in urinary metabolite concentration in children ages 6–17 years	Current asthma OR 0.26 (0.14, 0.49) Current wheeze OR 0.58 (0.24, 1.42) Current hay fever OR 0.78 (0.18, 3.48) Current rhinitis OR 1.52 (0.86, 2.66)	
			ΣDEHP		54.15–230.02 (survey-weighted)
			MEHP		<LOD–6.74
			MEHHP		15.16–75.70

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Table 2-8. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
			MEOHP 10.95–46.08	NR
			MECPP 25.29–101.93	NR
			OR for current allergic symptoms per log-unit increase in urinary metabolite concentration in adults (≥18 years)	
			ΣDEHP 33.21–160.81 (survey-weighted)	Current asthma OR 1.16 (0.82, 1.64) Current wheeze OR 1.23 (0.86, 1.77) Current hay fever OR 1.09 (0.59, 2.01) Current rhinitis OR 1.09 (0.86, 1.38)
			MEHP <LOD–6.19	NR
			MEHHP 9.59–50.46	NR
			MEOHP 6.10–13.48	NR
			MECPP 15.29–74.83	NR
Hsu et al. 2012, Cross-sectional (Taiwan)	101 children (63 boys, 38 girls), mean age 7 years; members of group recruited between 2005 and 2006 from randomly selected kindergartens and day care centers in Taiwan; from group, 59 cases with at least 2 parent-reported allergic disease or symptoms in prior 12 months and 42 controls selected; case status confirmed by clinical diagnosis. Urine samples collected during household visit for dust collection, within 1 year of clinical examination.	Logistic regression adjusted for child's gender, age, presence of fever, and if taken any medication in the recorded week; as well as parents' smoking status, allergic history and education levels, and the month the sampling took place	OR for allergic disease or symptom comparing highest quartile of urinary metabolite concentration with lowest	
			MEHP 5.7–20.0 µg/g Cr	Asthma OR 1.29 (0.13–12.89) Rhinitis OR 0.96 (0.25–3.71) Eczema OR 0.85 (0.20–3.55)
			MEHHP 25.4–85.3 µg/g Cr	NR
			MEOHP 23.4–79.6 µg/g Cr	NR

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Table 2-8. Selected Epidemiological Studies of DEHP Exposure and Allergy, Asthma, and Atopy

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Just et al. 2012, Cohort (United States [New York])	244 children (119 boys and 125 girls), members of CCCEH described above for Whyatt et al. (2014). Fractional exhaled NO concentrations (FeNO; a marker of airway inflammation) measured during followup visits when children were aged 4.9–9.1 years.	Generalized estimating equations with robust standard errors, adjusted for maternal urinary SG, age, sex, race/ethnicity, time of day of FeNO collection, and ambient NO, as well as seroatopy	Percent difference in fractional NO concentration with log-unit increase in maternal urinary metabolite concentration MEHHP 42 (36, 49) (GM [95% CI])	0.0 (-6.4, 6.9)

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

^bDemoralization is defined as “a psychological state characterized by helplessness, hopelessness, a sense of failure and the inability to cope” or a “giving up-given up” complex (Tecuta et al. 2015); maternal demoralization was assessed via questionnaire.

ΣDEHP = sum DEHP metabolites; BMI = body mass index; BPA = bisphenol A; CCCEH = Columbia Center for Children’s Environmental Health; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; GSD = geometric standard deviation; IgE = immunoglobulin E; IL-33 = interleukin 33; INMA = Infancia y Medio Ambiente; IQR = interquartile range; 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; NS = not significant; OR = odds ratio; RR = risk ratio; SE = standard error; SG = specific gravity; SG-adj = specific gravity adjusted; TSLP = thymic stromal lymphopoietin

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al. 2015a; Stelmach et al. 2015; Wang et al. 2014). In addition, although the data are more limited, no association was observed between maternal levels of urinary DEHP metabolites and food allergy in a small birth cohort study in Poland (n=147; Stelmach et al. 2015). Maternal urinary MEHHP levels during pregnancy were not associated with a change in fractional exhaled nitric oxide (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.

Animal Studies—Immune Function. Several animal studies have reported adjuvant effects of low levels of DEHP exposure in mice sensitized to OVA. In these studies, OVA-sensitized mice were exposed to DEHP prior to an OVA challenge. Immune responses were measured in treated animals and compared with responses in OVA-sensitized controls. 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. Enhanced immune responses in OVA-sensitized mice were also observed following oral exposure to DEHP doses ≥ 0.03 mg/kg/day (lowest dose tested) for 28–52 days (Guo et al. 2012; Han et al. 2014; Yang et al. 2008). Immune changes following OVA challenge were increased, including OVA-specific serum IgE and IgG1, cytokine production, and follicular helper cell population. Additionally, these researchers 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. At DEHP doses ≥ 0.7 mg/kg/day, there were increased eosinophils in BAL fluid and airway responsiveness (Guo et al. 2012; Han et al. 2014; Yang et al. 2008). 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. In the study by Guo et al. (2012), no exposure-related findings were observed in non-sensitized animals. Neither Han et al. (2014) nor Larsen et al. (2007) evaluated 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

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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.

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 $\geq 6,922$ mg/kg/day for 28 days; no changes occurred at doses $\leq 2,579$ 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), or chronic-duration studies at doses up to 1,821 mg/kg/day (Kluwe et al. 1982a, 1985, 1982b; 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). Additionally, 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

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changes in spleen, lymph node, and/or bone marrow histology or weights were observed in 30 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. 2014). These cells synthesize excesses of IL-21 and IL-4, which result in increased secretion of allergy-related IgE and IgG1. Overexpressed transcription factors related to this process include Bcl-6 and c-Maf (Han et al. 2014). DEHP also enhanced the production and/or secretion of tumor necrosis factor- α (TNF α) 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). Five cross-sectional studies have evaluated 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 adult (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).

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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). However, Shiue (2015c), in an analysis of 5,560 adult (20–80 years of age) NHANES (2011–2012) participants, observed an association between risk of depression and increased concentrations of MECPP in urine (OR 1.22; 95% CI 1.00, 1.48), but not other DEHP metabolites. Furthermore, 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.

Wang et al. (2015) reported clinical symptoms of neurotoxicity (i.e., headache, fatigue, dizziness, muscle weakness) in Chinese workers exposed to DEHP at three different PVC manufacturing facilities (average exposures ranging between 233 and 707 $\mu\text{g}/\text{m}^3$ 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 animals 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.

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(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. In a 1-generation study in mice, no changes in exploratory behavior were observed in F0 animals after 3 weeks of exposure to doses up to 180.77 mg/kg/day (behavior assessed 1 week prior to mating) (Tanaka 2002). Clinical signs of neurotoxicity were reported in mice exposed to $\geq 6,922$ mg/kg/day 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 21 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 are extremely limited. Based on available animal data, the adult neurological system is not a sensitive target of DEHP neurotoxicity.

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 in adult animals are below. Several studies evaluating potential mechanisms of reproductive toxicity are also discussed.

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Epidemiology Studies—Male Reproductive Effects. Cross-sectional studies examining serum testosterone levels in men have indicated associations between decreasing total and/or free testosterone levels and increasing urinary MEHP levels (Chang et al. 2015; Joensen et al. 2012; Jurewicz et al. 2013; Meeker et al. 2009a; Mendiola et al. 2012; Pan et al. 2006; Wang et al. 2016); associations with other metabolites have not been seen (Table 2-9). The association was seen in studies of men recruited from the general population (Joensen et al. 2012) as well as among male partners of subfertile couples (Jurewicz et al. 2013; Meeker et al. 2009a; Wang et al. 2016) and in PVC workers with high exposure levels (MEHP urinary levels between 210 and 1,884 µg/g creatinine [interquartile range]; Pan et al. 2006). Among studies that did not observe any association with serum testosterone (Axelsson et al. 2015; Fong et al. 2015; Jönsson et al. 2005; Meeker and Ferguson 2014; Mendiola et al. 2011; Pan et al. 2015), two (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 other reproductive hormone levels in serum were also observed in males in several of these cross-sectional studies. Reduced serum estradiol was associated with increased urinary MEHP in four studies (Meeker et al. 2009a; Mendiola et al. 2012; Pan et al. 2015; Wang et al. 2016), and increased sex hormone-binding globulin (SHBG) was associated with increased urinary levels of MEHP (Mendiola et al. 2011), MEOHP (Chang et al. 2015; Mendiola et al. 2012), and MEHHP (Mendiola et al. 2012). None of the studies observed a relationship with luteinizing hormone (LH) or inhibin B, and 11 of the 12 studies that evaluated serum FSH observed no association with DEHP metabolites in urine.

Only two 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) observed an inverse association between INSL-3 and urinary MEHP, while Chang et al. (2015) saw no relationship with any DEHP metabolite.

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Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Coefficient (β) (95% CI) unless otherwise specified ^a	
General population studies					
Axelsson et al. 2015, Cross-sectional (Sweden)	314 men, ages 17–20, residing within 60 km of Malmö, Sweden; participant and mother born and raised in Sweden; recruited between 2008 and 2010 from general population. Urine and blood samples collected at baseline visit.	Regression adjusted for BMI, own and parental smoking, and time of day	Mean difference in serum hormone level between highest and lowest quartiles of Cr-adjusted urinary metabolite concentration		
			MEHP	0.01–19 nmol/mmol Cr (min–max)	TT -2.7 (-11, 6.7) FT -0.9 (-8.8, 7.7) E2 4.8 (-4.2, 15) SHBG -1.3 (-4.7, 2.1) LH 6.3 (-4.7, 19) FSH 3.3 (-12, 22)
			MEHHP	0.5–340 nmol/mmol Cr	TT -0.9 (-9.7, 8.7) FT 1.1 (-6.9, 10) E2 2.3 (-6.5, 12) SHBG -1.5 (-4.9, 2.0) LH -4.8 (-15, 6.3) FSH -10 (-24, 5.9)
			MEOHP	0.2–200 nmol/mmol Cr	TT -1 (-9.7, 8.7) FT 0.4 (-7.7, 9.2) E2 0.8 (-7.9, 10) SHBG -1.0 (-4.4, 2.4) LH -7.2 (-17, 3.6) FSH -9.1 (-23, 7.1)
			MECPP	0.3–110 nmol/mmol Cr	TT 4.7 (-4.5, 15) FT 2.7 (-5.4, 12) E2 4.4 (-4.6, 14) SHBG 2.1 (-1.3, 5.6) LH -6.9 (-17, 4.0) FSH -8.9 (-23, 7.3)

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Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Coefficient (β) (95% CI) unless otherwise specified ^a	
Meeker and Ferguson 2014, Cross-sectional (United States)	867 males including 160 ages 12–20, 267 ages 20–40, 221 ages 40–60, and 219 ages 60–80; participants in NHANES 2011–2012. Urine and blood samples collected same day.	Linear regression adjusted for urinary Cr, age, poverty-income ratio, BMI, race/ethnicity, and session of sample collections	Percent change in serum total testosterone (ng/dL) with IQR increase in Cr-adj urinary metabolite concentration		
			Σ DEHP	NR	-8.42 (-24.5, 11.0)
				Ages 12–20	0.42 (-5.84, 7.09)
				Ages 20–<40	-7.84 (-15.8, 0.85)
				Ages 40–<60	-4.86 (-17, 9.01)
				Ages 60–80	
			MEHP	0.73–2.79	-9.23 (-27.1, 13.0)
				Ages 12–20	1.16 (-6.26, 9.17)
				Ages 20–<40	-8.48 (-17.5, 1.57)
				Ages 40–<60	0.81 (-13.2, 17.1)
				Ages 60–80	
			MEHHP	4.83–11.9	-2.63 (-19.5, 17.8)
				Ages 12–20	0.69 (-5.17, 6.91)
				Ages 20–<40	-7.30 (-14.7, 0.76)
				Ages 40–<60	-1.78 (-13.8, 11.9)
				Ages 60–80	
MEOHP	3.04–7.41	-7.61 (-24.7, 13.3)			
	Ages 12–20	1.29 (-5.29, 8.34)			
	Ages 20–<40	-7.93 (-15.9, 0.84)			
	Ages 40–<60	-6.24 (-18.9, 8.4)			
	Ages 60–80				
MECPP	7.97–21.6	-10.9 (-26.5, 7.99)			
	Ages 12–20	0.20 (-5.64, 6.4)			
	Ages 20–<40	-7.21 (-15.3, 1.61)			
	Ages 40–<60	-6.61 (-18.5, 7.06)			
	Ages 60–80				

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Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Coefficient (β) (95% CI) unless otherwise specified ^a	
Joensen et al. 2012, Cross-sectional (Denmark)	881 young Danish men from the general population recruited during 2007–2009 (mean age 19.5 years; range ~18–22 years). Urine and blood samples collected same day.	Linear regression adjusted for age, BMI, smoking, alcohol intake, and time of day of blood sample	Regression coefficient for difference in ln-transformed hormone level between the highest and lowest quartiles of % MEHP		
			Σ DEHP	15–260 (5 th –95 th percentile) [2.9–17% MEHP, 5 th –95 th percentile]	NR
			MEHP	0.4–18	TT -0.07 (-0.13, -0.01)* FT -0.07 (-0.12, -0.003)* E2 0.01 (-0.05, 0.07) SHBG 0.01 (-0.06, 0.09) LH 0.01 (-0.07, 0.10) FSH -0.14 (-0.25, -0.03)* Inhibin-B -0.02 (-0.09, 0.06)
			MEHHP	4.3–79	NR
			MEOHP	2.4–55	NR
MECPP	3.0–54	NR			
Jönsson et al. 2005, Cross-sectional (Sweden)	234 men ages 18–21 (28% smokers) living within 60 km of Malmö, recruited at medical conscript examination in 2000; urine and serum samples collected at examination.	Linear regression (abstinence time and smoking considered as covariates but not included in final model)	Mean difference in serum hormone level between highest and lowest quartiles of Cr-adjusted urinary phthalate metabolite concentration		
			MEHP	<LOD–5.1 nmol/mmol Cr	TT 0.8 (-1.1, 2.7) E2 -0.6 (-6.3, 5.2) SHBG 3.1 (-0.3, 6.4) LH 0.1 (-0.4, 0.6) FSH 0.2 (-0.3, 0.8) Inhibin B 4.9 (-16, 26)

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Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Coefficient (β) (95% CI) unless otherwise specified ^a	
Populations recruited from fertility clinics					
Mendiola et al. 2012 Cross-sectional (United States [California, Massachusetts, Minnesota, Missouri, New York, Iowa])	850 men; 425 male partners (mean age 32.2 years) of pregnant women who conceived without assistance, recruited at prenatal clinics in five U.S. cities between 1999 and 2005 (subset of the Study for Future Families multicenter study; Mendiola et al. 2011) and 425 male partners (mean age 36 years) of infertile couples seeking fertility evaluation at Massachusetts General Hospital between January 2000 and May 2004 (Meeker et al. 2009a). Urine and blood samples collected at baseline.	Linear regression adjusted for age, age squared, BMI, smoking status, ethnicity, study center time of sample collection, time of collection squared, and urinary dilution (Cr or specific gravity)	Association between log-transformed serum hormone level and log-transformed urinary metabolite concentration in fertile and infertile men (combined)		
			MEHP	0.9–39.2 (10 th –90 th percentile)	TT -0.01 (-0.03, 0.005) FT -0.02 (-0.04, -0.004)* E2 -7.9 (-12.4, -3.5)* SHBG 0.01 (-0.01, 0.03) LH -0.01 (-0.03, 0.01) FSH 0.01 (-0.01, 0.04)
			MEHHP	5.4–170	TT -0.01 (-0.03, 0.02) FT -0.02 (-0.04, -0.001)* E2 -3.4 (-8.8, 1.9) SHBG 0.03 (0.002, 0.06)* LH -0.02 (-0.05, 0.01) FSH -0.01 (-0.04, 0.03)
			MEOHP	3.2–110	TT -0.01 (-0.03, 0.02) FT -0.02 (-0.04, -0.001)* E2 -3.0 (-8.4, 2.3) SHBG 0.03 (0.005, 0.06)* LH -0.02 (-0.05, 0.01) FSH -0.01 (-0.05, 0.02)
Among fertile men (Mendiola et al. 2011), a significant positive association was seen between MEHP and SHBG, but not for other metabolites or hormones. Among infertile men, significant negative associations were seen between SG-adjusted MEHP and total testosterone and estradiol levels (Meeker et al. 2009a).					

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Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Coefficient (β) (95% CI) unless otherwise specified ^a	
Wang et al. 2016, Cross-sectional (China)	1,040 male partners of couples attending infertility clinic in Wuhan China. Two urine samples at least 2 hours apart and one blood sample were collected.	Linear regression, adjusted for age, BMI, alcohol use, smoking status, daily cigarette consumption, and urinary Cr	Association between in serum hormone level and ln-transformed Cr-adjusted urinary metabolite concentration		
			MEHP	1 st sample: 2.37–7.35 $\mu\text{g/g Cr}$ 2 nd sample: 2.53–8.80 $\mu\text{g/g Cr}$	Significant ($p < 0.05$) associations between \downarrow TT, FT, and E2 and \uparrow MEHP in urine.
			MEHHP	1 st sample: 6.80–15.07 $\mu\text{g/g Cr}$ 2 nd sample: 6.86–16.70 $\mu\text{g/g Cr}$	
MEOHP	1 st sample: 3.91–8.45 $\mu\text{g/g Cr}$ 2 nd sample: 3.94–9.27 $\mu\text{g/g Cr}$	No significant association between MEHP and FSH or LH, or between MEHHP and MEOHP and any hormone level (data shown graphically)			
Chang et al. 2015, Case-control (Taiwan)	176 men (25–45 years) recruited between 2010 and 2012, including infertile men (n=141) recruited through infertility clinics in Taiwan and fertile men (n=35) recruited from childbirth preparation classes. 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). Urine and blood samples collected same day.	Linear regression adjusted for age, BMI, cigarettes/day, and season during which blood was collected; TT and E2 also adjusted for SHBG.	Change in ln-transformed serum hormone level per IQR increase in ln-transformed Cr-adjusted urinary metabolite concentration		
			Σ DEHP	Fertile 0.11 (0.07) $\mu\text{mol/g Cr}$ Infertile 1 0.12 (0.06) $\mu\text{mol/g Cr}$ Infertile 2 0.14 (0.15) $\mu\text{mol/g Cr}$ GM (GSD)	TT 0.98 (0.91, 1.06) FT 0.95 (0.79, 1.14) E2 0.99 (0.90, 1.09) SHBG 1.05 (0.95, 1.16) LH 1.05 (0.95, 1.17) FSH 1.03 (0.91, 1.16) Inhibin B 1.00 (0.87, 1.15) INSL3 1.07 (0.94, 1.21)
			MEHP	Fertile 3.21 (0.30) $\mu\text{g/g Cr}$ Infertile 1 4.11 (0.28) $\mu\text{g/g Cr}$ Infertile 2 4.52 (0.33) $\mu\text{g/g Cr}$	TT 0.93 (0.87, 0.99)* FT 0.84 (0.71, 0.99)* E2 0.99 (0.91, 1.09) SHBG 1.00 (0.90, 1.10) LH 1.06 (0.96, 1.16) FSH 1.03 (0.92, 1.16) Inhibin B 1.00 (0.88, 1.14) INSL3 0.93 (0.83, 1.05)

2. HEALTH EFFECTS

Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Coefficient (β) (95% CI) unless otherwise specified ^a
			MEHHP Fertile 8.30 (0.79) $\mu\text{g/g Cr}$	TT 1.00 (0.94, 1.07)
			Infertile 1 9.94 (0.70) $\mu\text{g/g Cr}$	FT 1.06 (0.89, 1.25)
			Infertile 2 10.1 (0.78) $\mu\text{g/g Cr}$	E2 0.99 (0.91, 1.08)
				SHBG 1.04 (0.95, 1.14)
				LH 1.08 (0.98, 1.18)
				FSH 1.05 (0.95, 1.08)
				Inhibin B 0.97 (0.85, 1.10)
				INSL3 1.06 (0.95, 1.19)
			MEOHP Fertile 6.14 (0.72) $\mu\text{g/g Cr}$	TT 0.99 (0.93, 1.06)
			Infertile 1 5.85 (0.39) $\mu\text{g/g Cr}$	FT 1.01 (0.85, 1.21)
Infertile 2 5.66 (0.38) $\mu\text{g/g Cr}$	E2 0.99 (0.90, 1.09)			
	SHBG 1.09 (1.01, 1.19)*			
	LH 1.05 (0.95, 1.16)			
	FSH 1.05 (0.94, 1.18)			
	Inhibin B 1.01 (0.89, 1.15)			
	INSL3 1.06 (0.99, 1.30)			
			MECPP Fertile 9.15 (1.01) $\mu\text{g/g Cr}$	TT 0.99 (0.93, 1.06)
			Infertile 1 11.9 (0.83) $\mu\text{g/g Cr}$	FT 0.91 (0.77, 1.08)
			Infertile 2 12.4 (0.85) $\mu\text{g/g Cr}$	E2 1.01 (0.92, 1.10)
				SHBG 1.04 (0.95, 1.15)
				LH 1.04 (0.94, 1.14)
				FSH 0.99 (0.89, 1.11)
	Inhibin B 1.02 (0.89, 1.16)			
	INSL3 1.05 (0.93, 1.18)			

2. HEALTH EFFECTS

Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Coefficient (β) (95% CI) unless otherwise specified ^a	
Pan et al. 2015, Cross-sectional (China)	1,066 male partners (mean age 29.1 years) of infertile couples recruited from the Reproductive Medical Center at Nanjing Jinling Hospital in Nanjing, China, between November 2012 and July 2014. Urine and nonfasting blood samples collected at recruitment.	Linear regression adjusted for age, BMI, smoking, drinking, time of blood draw, and urinary Cr	Percent change in ln-transformed serum hormone level with IQR increase in ln-transformed urinary metabolite concentration		
			MEHP	2.4–8.7	TT -1.2 (-3.5, 1.1) E2 -4.3% (-8.2, -0.2%)* SHBG 0.9% (-2.0, 3.8%) LH -0.2% (-3.5, 3.3%) FSH 3.1% (-3.4, 4.2%) INSL3 -4.3% (-7.7, -0.8%)*
			MEHHP	7.6–22.5	NR
			MEOHP	4.8–14.3	NR
Jurewicz et al. 2013, Cross-sectional (Poland)	269 men <45 years of age (mean age 32 years) attending infertility clinic for diagnostic purposes, with sperm concentration $\geq 15 \times 10^6/\text{mL}$. Urine, semen, and blood samples collected same day.	Linear regression adjusted for age, smoking, abstinence period, past diseases, and urinary Cr	Regression coefficient for association between serum hormone level and log-transformed urinary metabolite concentration		
			MEHP	0.5–399.3 $\mu\text{g/g Cr}$ (min–max)	TT -0.29* (NR) E2 0.14 (NR) FSH 0.08 (NR)
			MEOHP	1.2–131.0 $\mu\text{g/g Cr}$	TT 0.16 (NR) E2 -0.06 (NR) FSH 0.12 (NR)

2. HEALTH EFFECTS

Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Coefficient (β) (95% CI) unless otherwise specified ^a	
Occupationally exposed populations					
Fong et al. 2015, Occupational (Taiwan)	82 male PVC production workers (mean age 38 years) who worked for at least 1 year at one of three Taiwanese plants using only DEHP as plasticizer. Urine and blood samples collected on the last day of each subject's work week.	Linear regression adjusted for age, seniority in current job, BMI, current smoking and drinking (within 1 month), and SHBG level	Association between ln-transformed serum hormone level and log-transformed post-shift Cr-adjusted urinary metabolite concentration		
			MEHP	11.5–36.0 $\mu\text{g/g Cr}$	TT 0.041 (NR) E2 0.153 (NR) SHBG -0.019 (NR) LH 0.014 (NR) FSH 0.015 (NR) Inhibin B -0.003 (NR)
			MEHHP	46.2–150.5 $\mu\text{g/g Cr}$	TT 0.035 (NR) E2 0.160 (NR) SHBG -0.035 (NR) LH 0.018 (NR) FSH -0.001 (NR) Inhibin B 0.029 (NR)
			MEOHP	38.8–111.3 $\mu\text{g/g Cr}$	TT 0.055 (NR) E2 0.163 (NR) SHBG -0.014 (NR) LH 0.046 (NR) FSH 0.009 (NR) Inhibin B 0.031 (NR)

2. HEALTH EFFECTS

Table 2-9. Summary of Epidemiological Studies of DEHP Exposure and Serum Reproductive Hormones or Leydig Cell Functionality in Adult Men

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Coefficient (β) (95% CI) unless otherwise specified ^a
Pan et al. 2006, Occupational (China)	74 exposed male PVC workers (mean age 33.5 years) and 63 unexposed male construction workers (mean age 34.3 years). Urine and blood samples collected on same day (not first work day of week or day after night shift).	Linear regression (age and alcohol intake, evaluated in separate models, were also significantly associated with free testosterone)	Association between log-transformed serum hormone level and log-transformed urinary metabolite concentration MEHP Exposed: 209.6–1,884.4 $\mu\text{g/g Cr}$ Unexposed: 3.7–9.9 $\mu\text{g/g Cr}$	FT -0.235* (NR) No association with E2, LH, or FSH

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

Σ DEHP = sum DEHP metabolites; BMI = body mass index; CI = confidence interval; Cr = creatinine; Cr-adj = creatinine-adjusted; DEHP = di(2-ethylhexyl)-phthalate; 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; 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; min = minimum; NHANES = National Health and Nutrition Examination Survey; NR = not reported; PVC = polyvinyl chloride; 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

2. HEALTH EFFECTS

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 2-10. Most of the studies evaluating sperm morphology suggested potential weak associations between exposure to DEHP and increased odds of sperm morphology below the World Health Organization (WHO) reference value for normal morphology (Han et al. 2014; Herr et al. 2009; Wirth et al. 2008) or a lower percent normal sperm with increasing DEHP exposure (Axelsson et al. 2015; Bloom et al. 2015a, 2015b; Huang et al. 2014b).

A negative relationship was suggested between reduced sperm count and/or concentration and DEHP metabolites in urine in most studies evaluating these endpoints (Axelsson et al. 2015; Bloom et al. 2015a, 2015b; Han et al. 2014; Hauser et al. 2006; Herr et al. 2009; Huang et al. 2014b; Jurewicz et al. 2013; Pan et al. 2015; Wirth et al. 2008). When percent motile sperm was evaluated as a continuous variable, negative relationships were reported in five (Axelsson et al. 2015; Bloom et al. 2015a, 2015b; Huang et al. 2014b; Jurewicz et al. 2013; Pan et al. 2015) of seven studies. In contrast, most studies that dichotomized percent motile sperm (above and below WHO reference values) reported reduced odds of low motility sperm (Table 2-10).

The extent to which effects on semen quality affect fertility in exposed men has not been well-studied. In the only identified prospective cohort study evaluating potential associations between time-to-pregnancy and urinary metabolite levels (n=439 couples), DEHP exposure in men was not associated with increased time to pregnancy; fecundability-adjusted odds ratios did not differ from 1.0 ($p>0.05$) for urinary levels of MECPP (FOR 0.89, 95% CI 0.77–1.03), MEHHP (FOR 0.93, 95% CI 0.82–1.07), MEOHP (FOR 0.91, 95% CI 0.79–1.05), or MEHP (FOR 0.98, 95% CI 0.87–1.10) (Buck Louis et al. 2014).

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).

2. HEALTH EFFECTS

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Sperm Morphology or Motility

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Sperm morphology					
Axelsson et al. 2015, Cross-sectional (Sweden)	314 men, ages 17–20, residing within 60 km of Malmö, Sweden; participant and mother born and raised in Sweden; recruited between 2008 and 2010 from general population. Urine and semen samples collected at baseline visit.	Regression adjusted for abstinence time, BMI, and own and parental smoking	Mean difference in percent normal sperm between highest and lowest quartiles of Cr-adjusted urinary metabolite concentration		
			MEHP	0.01–19 nmol/mmol Cr (min–max)	Difference = -1.3 (-3.3, 0.66)
			MEHHP	0.5–340 nmol/mmol Cr	Difference = -1.2 (-3.2, 0.8)
			MEOHP	0.2–200 nmol/mmol Cr	Difference = -0.42 (-2.4, 1.6)
			MECPP	0.3–110 nmol/mmol Cr	Difference = -1.0 (-3.0, 0.98)
Bloom et al. 2015a, 2015b, Cohort (United States [Michigan, Texas])	473 male partners (ages 19–51 years; mean age 31.8 years) of couples trying to conceive after discontinuing contraception; participants in the LIFE cohort; recruited from general population of 16 counties in Michigan and Texas from 2005 to 2009. Urine samples were collected at baseline, and semen samples collected at baseline and 1 month later.	Linear regression adjusted for age, race, BMI, income, serum cotinine, urine Cr, abstinence time, and study site	Difference in Box-Cox-transformed percent normal sperm (WHO criteria; baseline semen sample only) per IQR increase in ln-transformed urinary metabolite concentration		
			MEHP	0 ^b –4.87	β -0.78 (-13.29, 11.72)
			MEHHP	5.56–37.94	β -4.33 (-12.89, 4.23)
			MEOHP	3.06–17.9	β -3.35 (-11.36, 4.67)
			MECPP	8.60–46.4	β -3.37 (-12.61, 5.86)

2. HEALTH EFFECTS

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Sperm Morphology or Motility

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Pan et al. 2015, Cross-sectional (China)	1,066 male partners (median age 29.1 years) of infertile couples recruited from the Reproductive Medical Center at Nanjing Jinling Hospital in Nanjing, China, between November 2012 and July 2014. Urine and semen samples collected at recruitment.	Linear regression adjusted for age, BMI, smoking, drinking, abstinence time, and urinary Cr	Percent change in percent normal sperm associated with IQR increase in ln-transformed urinary metabolite concentration		
			MEHP	2.4–8.7	β -0.7 (-3.7, 2.4)
			MEHHP	7.6–22.5	NR
			MEOHP	4.8–14.3	NR
Huang et al. 2014b, Occupational (Taiwan)	47 exposed male PVC workers <60 years of age and 15 unexposed male graduate students (mean age 25.3 years); the exposed group was further divided into those expected to have high exposure (n=36, mean age 35.5 years, direct contact with DEHP in manufacturing process) and those expected to have low exposure (n=11, mean age 36.3 years, administrative, sales, or guard officers). Urine and semen samples collected same day.	Multiple linear regression adjusted for age, smoking status, and coffee consumption	Regression coefficient for association between percent normal sperm morphology and Cr-adjusted urinary metabolite concentration		
			MEHP	Control: 4.4–13.5 $\mu\text{g/g Cr}$ Low: 9.2–21.4 $\mu\text{g/g Cr}$ High: 11.5–31.9 $\mu\text{g/g Cr}$	β 0.090 (-0.123, 0.304)
			MEHHP	Control: 13.0–28.5 $\mu\text{g/g Cr}$ Low: 29.7–54.2 $\mu\text{g/g Cr}$ High: 47.1–111.5 $\mu\text{g/g Cr}$	β -0.005 (-0.065, 0.055)
			MEOHP	Control: 10.1–19.9 $\mu\text{g/g Cr}$ Low: 20.4–48.5 $\mu\text{g/g Cr}$ High: 41.0–99.4 $\mu\text{g/g Cr}$	β -0.001 (-0.074, 0.071)

2. HEALTH EFFECTS

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Sperm Morphology or Motility

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Jurewicz et al. 2013, Cross-sectional (Poland)	269 men <45 years of age (mean age 32 years) attending infertility clinic for diagnostic purposes, with sperm concentration $\geq 15 \times 10^6$ /mL. Urine and semen samples collected same day.	Linear regression adjusted for age, smoking, abstinence period, past diseases, and urinary Cr	Regression coefficient for association between percent normal morphology sperm and log-transformed urinary metabolite concentration	
			MEHP	0.5–399.3 $\mu\text{g/g Cr}$ (min–max) β 1.29 (NR)
			MEOHP	1.2–131.0 $\mu\text{g/g Cr}$ β 1.31 (NR)
Joensen et al. 2012, Cross-sectional (Denmark)	881 young Danish men from the general population recruited during 2007–2009 (mean age 19.5 years; range ~18–22 years). Urine and semen samples collected same day.	Linear regression (no adjustments)	Regression coefficient for difference in square-root-transformed percent of morphologically normal sperm between the highest and lowest quartiles of percent MEHP	
			Σ DEHP	15–260 (5 th –95 th percentile) [2.9–17% MEHP, 5 th –95 th percentile] NR
			MEHP	0.4–18 β 0.11 (-0.08, 0.3)
			MEHHP	4.3–79 NR
			MEOHP	2.4–55 NR
			MECPP	3.0–54 NR
Han et al. 2014, Cross-sectional (China)	232 men between 20 and 40 years of age, with no known exposure to phthalate esters, recruited in 2007 at Chongqing Family Planning Research Institute and Reproductive Center as part of study on semen quality in general population (mean age 32 years).	Logistic regression adjusted for age and abstinence time	OR for percent normal sperm below the WHO reference value ($\geq 15\%$ normal) comparing those with Cr-adjusted urinary metabolite concentration greater than the median with those less than the median	
			MEHP	<LOD–31.4 $\mu\text{g/g Cr}$ (5 th –95 th percentile) OR 1.18 (0.58, 2.39)

2. HEALTH EFFECTS

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Sperm Morphology or Motility

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Herr et al. 2009, Cross-sectional (Germany)	349 male partners of subfertile couples recruited between April 2004 and November 2005 from the Centre of Dermatology and Andrology at the University Medical Center, Giessen Germany (mean age 34.2 years). Urine and semen samples collected same day.	Logistic regression adjusted for age, smoking, duration of abstinence, and urine Cr	OR for sperm morphology below the WHO reference value ($\geq 4\%$ normal morphology) comparing highest quartile of urinary metabolite concentration with lowest quartile		
			Σ DEHP	23.20–74.70	OR 1.95 (0.74, 5.16)
			MEHP	1.97–9.17	NR
			MEHHP	6.91–22.09	NR
			MEOHP	5.10–16.19	NR
Wirth et al. 2008, Cross-sectional (United States [Michigan])	45 male partners of subfertile couples seen for semen analysis at Michigan infertility clinic (mean age 34.8 years; timing of recruitment not reported). Urine and semen samples collected same day.	Multivariate logistic regression adjusted for specific gravity	OR for sperm morphology below the WHO reference value ($\geq 4\%$ normal morphology) comparing highest tertile of urinary metabolite concentration with lowest tertile		
			Σ DEHP	NA	OR 1.2 (0.3, 5.6)
			MEHP	4.6–22.1	NR
			MEHHP	32.7–137.1	NR
Hauser et al. 2006, Cross-sectional (United States [Massachusetts])	463 male partners (ages 20–54 years) of subfertile couples seen for semen analysis Massachusetts General Hospital between January 2000 and May 2004.	Multivariate logistic regression adjusted for age, abstinence time, and smoking	OR for sperm morphology below WHO reference value ($\geq 4\%$ normal morphology) comparing highest quartile of SG-adjusted urinary metabolite concentration with lowest quartile		
			MEHP	3.1–20.9	OR 0.7 (0.4, 1.5)
			MEHHP	23.4–113	OR 0.7 (0.3, 2.0)
(Update of Duty et al. 2003 with larger population size)	Urine and semen samples collected same day.		MEOHP	15.8–73.0	OR 0.7 (0.3, 2.0)

2. HEALTH EFFECTS

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Sperm Morphology or Motility

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Sperm motility (%)					
Axelsson et al. 2015, Cross-sectional (Sweden)	314 men, ages 17–20, residing within 60 km of Malmö, Sweden; participant and mother born and raised in Sweden; recruited between 2008 and 2010 from general population. Urine and semen samples collected at baseline visit.	Regression adjusted for abstinence time, BMI, and own and parental smoking	Mean difference in percent progressively motile sperm comparing highest and lowest quartiles of Cr-adjusted urinary metabolite concentration		
			MEHP	0.01–19 nmol/mmol Cr (min–max)	Difference = -7.8 (-14, -2)
			MEHHP	0.5–340 nmol/mmol Cr	Difference = -8.7 (-15, -2.8)
			MEOHP	0.2–200 nmol/mmol Cr	Difference = -6.9 (-13, -1.1)*
Bloom et al. 2015a, 2015b, Cohort (United States [Michigan, Texas])	473 male partners (ages 19–51 years; mean age 31.8 years) of couples trying to conceive after discontinuing contraception; participants in the LIFE cohort; recruited from general population of 16 counties in Michigan and Texas from 2005 to 2009. Urine samples were collected at baseline, and semen samples collected at baseline and 1 month later.	Linear regression adjusted for age, race, BMI, income, serum cotinine, urine Cr, abstinence time, and study site	Change in Box-Cox-transformed percent motile sperm per IQR increase in ln-transformed urinary metabolite concentration		
			MEHP	0 ^b –4.87	β 0.93 (-1.6, 3.46)
			MEHHP	5.56–37.94	β -1.46 (-3.18, 0.26)
			MEOHP	3.06–17.9	β -1.61 (-3.2, 0.00)*
			MECPP	8.60–46.4	β -1.88 (-3.73, -0.03)*

2. HEALTH EFFECTS

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Sperm Morphology or Motility

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Pan et al. 2015, Cross-sectional (China)	1,066 male partners (median age 29.1 years) of infertile couples recruited from the Reproductive Medical Center at Nanjing Jinling Hospital in Nanjing, China, between November 2012 and July 2014. Urine and semen samples collected at recruitment.	Linear regression adjusted for age, BMI, smoking, drinking, abstinence time, and urinary Cr	Percent change in progressively motile sperm (%) with IQR increase in ln-transformed urinary metabolite concentration		
			MEHP	2.4–8.7	β -2.1 (-4.9, 0.7)
			MEHHP	7.6–22.5	NR
			MEOHP	4.8–14.3	NR
Huang et al. 2014b, Occupational (Taiwan)	47 exposed male PVC workers <60 years of age and 15 unexposed male graduate students (mean age 25.3 years); the exposed group was further divided into those expected to have high exposure (n=36, mean age 35.5 years, direct contact with DEHP in manufacturing process) and those expected to have low exposure (n=11, mean age 36.3 years, administrative, sales, or guard officers). Urine and semen samples collected same day.	Multiple linear regression adjusted for age, smoking status, and coffee consumption	Regression coefficient for association between percent motile sperm motility and Cr-adjusted urinary metabolite concentration		
			MEHP	Control: 4.4–13.5 $\mu\text{g/g Cr}$ Low: 9.2–21.4 $\mu\text{g/g Cr}$ High: 11.5–31.9 $\mu\text{g/g Cr}$	β -0.549 (-0.952, -0.146)*
			MEHHP	Control: 13.0–28.5 $\mu\text{g/g Cr}$ Low: 29.7–54.2 $\mu\text{g/g Cr}$ High: 47.1–111.5 $\mu\text{g/g Cr}$	β -0.155 (-0.267, -0.043)*
			MEOHP	Control: 10.1–19.9 $\mu\text{g/g Cr}$ Low: 20.4–48.5 $\mu\text{g/g Cr}$ High: 41.0–99.4 $\mu\text{g/g Cr}$	β -0.201 (-0.336, -0.066)*

2. HEALTH EFFECTS

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Sperm Morphology or Motility

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Jurewicz et al. 2013, Cross-sectional (Poland)	269 men <45 years of age (mean age 32 years) attending infertility clinic for diagnostic purposes, with sperm concentration $\geq 15 \times 10^6$ /mL. Urine and semen samples collected same day.	Linear regression adjusted for age, smoking, abstinence period, past diseases, and urinary Cr	Regression coefficient for association between percent motile sperm and log-transformed urinary metabolite concentration	
			MEHP	0.5–399.3 $\mu\text{g/g Cr}$ (min–max) β -3.85* (NR)
			MEOHP	1.2–131.0 $\mu\text{g/g Cr}$ β -3.94* (NR)
Joensen et al. 2012, Cross-sectional (Denmark)	881 young Danish men from the general population recruited during 2007–2009 (mean age 19.5 years; range ~18–22 years). Urine and semen samples collected same day.	Linear regression adjusted for time to semen analysis	Regression coefficient for difference in squared percent of progressively motile sperm between the highest and lowest quartiles of percent MEHP	
			Σ DEHP	15–260 (5 th –95 th percentile) [2.9–17% MEHP, 5 th –95 th percentile] NR
			MEHP	0.4–18 β 289 (-40, 617)
			MEHHP	4.3–79 NR
			MEOHP	2.4–55 NR
Jönsson et al. 2005, Cross-sectional (Sweden)	234 men ages 18–21 (28% smokers) living within 60 km of Malmö, recruited at medical conscript examination in 2000; urine and semen samples collected at examination.	Linear regression (abstinence time and smoking considered as covariates but not included in final model)	Mean difference in sperm motility (%) between the highest and lowest quartiles of Cr-adjusted urinary metabolite concentration	
			MEHP	<LOD–12 nmol/mmol Cr Difference = 0.1 (-5.8, 6.1)

2. HEALTH EFFECTS

Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Sperm Morphology or Motility

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Han et al. 2014, Cross-sectional (China)	232 men between 20 and 40 years of age, with no known exposure to phthalate esters, recruited in 2007 at Chongqing Family Planning Research Institute and Reproductive Center as part of study on semen quality in general population (mean age 32 years).	Logistic regression adjusted for age and abstinence time	OR for percent motile sperm below the WHO reference values (grade A+B ≥50% or grade A ≥25%) comparing those with Cr-adjusted urinary metabolite concentration greater than the median with those less than the median	
			MEHP	<LOD–31.4 µg/g Cr (5 th –95 th percentile) OR 0.48 (0.08, 2.76)
Liu et al. 2012, Cross-sectional (China)	97 male partners of subfertile couples (mean age 31.5 years) seeking fertility assessment at The Reproduction Department of the Chongqing Institute of Science and Technology for Population and Family Planning from July 2009 to August 2010. Urine and semen samples collected same day.	Multivariate logistic regression adjusted for age, BMI, abstinence time, smoking, and drinking behavior	OR for percent motile sperm below WHO reference value (≥50% motile) comparing highest tertile of urinary metabolite concentration, with lowest tertile	
			MEHP	0.35–1.93 µg/g Cr (33 rd –66 th percentile) OR 0.8 (0.3, 2.4)
			MEOHP	1.89–3.05 µg/g Cr OR 0.6 (0.2, 1.8)
Herr et al. 2009, Cross-sectional (Germany)	349 male partners of subfertile couples recruited between April 2004 and November 2005 from the Centre of Dermatology and Andrology at the University Medical Center, Giessen Germany (mean age 34.2 years). Urine and semen samples collected same day.	Logistic regression adjusted for age, smoking, duration of abstinence, and urine Cr	OR for sperm motility below the WHO reference value (>50% motile) comparing highest quartile of urinary metabolite concentration with lowest quartile	
			ΣDEHP	23.20–74.70 OR 0.86 (0.26, 2.86)
			MEHP	1.97–9.17 NR
			MEHHP	6.91–22.09 NR
			MEOHP	5.10–16.19 NR
			MECPP	8.03–27.23 NR

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Table 2-10. Summary of Epidemiological Studies of DEHP Exposure and Sperm Morphology or Motility

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)		Effect estimate (95% CI) ^a
Wirth et al. 2008, Cross-sectional (United States [Michigan])	45 male partners of subfertile couples seen for semen analysis at Michigan infertility clinic (mean age 34.8 years; timing of recruitment not reported). Urine and semen samples collected same day.	Multivariate logistic regression adjusted for age, servings per week of alcohol, and urinary specific gravity	OR for sperm motility below the WHO reference value (>50% motile) comparing highest tertile of urinary metabolite concentration with lowest tertile		
			ΣDEHP	NA	OR 0.7 (0.1, 4.4)
			MEHP	4.6–22.1	NR
			MEHHP	32.7–137.1	NR
Hauser et al. 2006, Cross-sectional (United States [Massachusetts])	463 male partners (ages 20–54 years) of subfertile couples seen for semen analysis Massachusetts General Hospital between January 2000 and May 2004. Urine and semen samples collected same day.	Multivariate logistic regression adjusted for age, abstinence time, and smoking	OR for sperm motility below WHO reference value (≥50% motile) comparing highest quartile of SG-adjusted urinary metabolite concentration with lowest quartile		
			MEHP	3.1–20.9	OR 1.1 (0.6, 1.9)
			MEHHP	23.4–113	OR 0.8 (0.4, 1.8)
			MEOHP	15.8–73.0	OR 0.8 (0.3, 1.6)

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

^bValue reported in study was less than zero, reflecting correction for analytical blank; adjusted to 0 for reporting in this table.

ΣDEHP = sum DEHP metabolites; BMI = body mass index; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; IQR = interquartile range; LIFE = Longitudinal Study of Infertility and Environment; 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; min = minimum; NA = not applicable; NR = not reported; OR = odds ratio; PVC = polyvinyl chloride; SG-adj = specific gravity-adjusted; WHO = World Health Organization

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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 $\geq 1,040$ 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 ($\geq 1,040$ 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 $\geq 5,000$ 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

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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.

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 $\geq 1,000$ mg/kg/day (Dostal et al. 1988). In 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, including testicular atrophy and degeneration, degeneration of the Leydig cells, decreased spermatogenesis/hypospermia, and decreased testicular weights, were observed at ≥ 300 mg/kg/day (CMA 1984; Exxon Chemical Americas 1990; Myers 1992b; NTP 1982; Shaffer et al. 1945). However, two intermediate-duration studies reported no histopathological changes in rat testes at doses up to 200 mg/kg/day for 28 days (Akingbemi et al. 2001) or 930 mg/kg/day for 3 weeks (Astill et al. 1986). 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. In A/J mice, Sertoli cell vacuolation and germ cell sloughing in seminiferous tubules were observed 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). However, testicular effects, including testicular atrophy, decreases/absent spermatogenesis, and decreased testes/epididymides weights, were observed in B6C3F1 mice after intermediate-duration exposure to doses $\geq 2,579$ mg/kg/day, but not $\leq 2,500$ mg/kg/day (Myers 1992a; NTP 1982). 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

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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 rats 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).

To evaluate the antiandrogenic potential of DEHP, Lee and Koo (2007) and Stroheker et al. (2005) conducted Hershberger Assays in Sprague-Dawley and Wistar rats, respectively. In these studies, male rats were castrated and subsequently supplemented with testosterone so control and exposed animals had equivalent testosterone levels. Following DEHP exposure 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. Similarly, Stroheker et al. (2005) observed significantly decreased LABC muscle weights at ≥ 100 mg/kg/day, decreased prostate weights at ≥ 200 mg/kg/day, and decreased seminal vesicles weights at ≥ 400 mg/kg/day; no exposure-related findings were observed at ≤ 20 mg/kg/day. As expected, no exposure-related changes in serum testosterone were observed in either study. Reproductive organ histology was not assessed. These studies indicate that DEHP exhibits some antiandrogenic activity.

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

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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. In a study in PVC workers, increased urinary DEHP metabolite levels were associated with both decreased sperm quality and sperm ROS generation (Huang et al. 2014b). Other 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).

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 steroidogenesis 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 steroidogenesis from ¹⁴C-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).

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Many of the available studies (Barrett et al. 2014; Buck Lewis et al. 2013; Grindler et al. 2015; Huang et al. 2010, 2014b; Itoh et al. 2009; Kim et al. 2015; Pollack et al. 2015; Sun et al. 2015, 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).

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; Table 2-11). One of these studies (Jukic et al. 2016) evaluated the menstrual cycle, observing that DEHP metabolites were not associated with altered follicular phase length. A cohort study of women (n=215) 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 2-11). 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 sex hormone binding globulin (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

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Table 2-11. Summary of Epidemiological Studies of DEHP Exposure and Female Reproductive Effects

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Thomsen et al. 2017, Cohort (Denmark)	229 women (ages 20–35 years) recruited between 1992 and 1995, for the Danish First Pregnancy Planner study. First morning urine samples collected from day 1 to 10 in each cycle until pregnancy achieved or 6 months passed. Urinary phthalate levels were measured in the first urine sample (day 1–10 of first cycle) and a second urine sample (the last taken prior to pregnancy or in the sixth cycle for women who did not become pregnant). Time of pregnancy was diagnosed by general practitioner.	Discrete-time Cox regression model adjusted for age, BMI, alcohol, and smoking	Fecundability odds ratio per ln unit increase in ln-transformed urinary metabolite concentration, conditional on not achieving pregnancy in previous cycle		
			MEHP	14.5 (0–348) (median [min–max])	0.99 (0.72, 1.35)
			MEHHP	NR	0.70 (0.48, 1.03)
			MEOHP	NR	0.83 (0.58, 1.18)
Buck Louis et al. 2014, Cohort (United States [Michigan, Texas])	454 women (ages 18–44) recruited when discontinuing contraception to become pregnant; recruited using population-based sampling from 16 counties in Michigan and Texas between 2005 and 2009 (members of Longitudinal Investigation of Fertility and the Environment cohort). Women completed daily journal regarding intercourse, menstruation, and home pregnancy tests; home fertility monitors that track rise in estrone-3-glucuronide and LH used to time intercourse. Single urine sample collected at baseline. Time to pregnancy determined by journals and fertility monitoring results.	Cox models for discrete survival time, adjusted for both partners' urinary metabolite and creatinine concentrations, age, BMI, serum cotinine, and research site	Fecundability odds ratio per SD increase in log-transformed and scaled (by SD) urinary metabolite concentration, conditional on not achieving pregnancy in previous cycle		
			MEHP	Pregnant: 4.56 (3.40–6.11) Not pregnant: 5.60 (3.81–8.24)	0.99 (0.87, 1.12)
			MEHHP	Pregnant: 15.24 (13.01–17.86) Not pregnant: 14.46 (11.52–18.14)	1.06 (0.91, 1.24)
			MEOHP	Pregnant: 8.65 (7.40–10.10) Not pregnant: 7.55 (5.86–9.74)	1.08 (0.92, 1.27)
			MECPP	Pregnant: 21.18 (18.25–24.58) Not pregnant: 21.21 (16.94–26.55)	1.06 (0.91, 1.24)

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Table 2-11. Summary of Epidemiological Studies of DEHP Exposure and Female Reproductive Effects

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a																		
Jukic et al. 2016, Cohort (United States [North Carolina])	221 healthy women recruited when discontinuing contraception to become pregnant, between 1982 and 1986, from communities in North Carolina (members of North Carolina Early Pregnancy Study). First morning urine samples collected daily until pregnancy achieved or 6 months passed; three stored samples per cycle were pooled for phthalate metabolite analysis. Time to pregnancy measured as numbers of ovulatory cycles (identified by urinary analysis for estrone 3-glucuronide and pregnenediol 3-glucuronide) between enrollment and conception.	Discrete-time, time-to-event model adjusted for age, age at menarche, current smoking, alcohol intake, BMI, caffeine consumption, and education	Fecundability ratio comparing highest and lowest tertiles of urinary metabolite concentrations <table border="1"> <thead> <tr> <th>Metabolite</th> <th>NR</th> </tr> </thead> <tbody> <tr> <td>ΣDEHP</td> <td></td> </tr> <tr> <td>MEHP</td> <td>3.8–11.2</td> </tr> <tr> <td>MEHHP</td> <td>31.8–80.8</td> </tr> <tr> <td>MEOHP</td> <td>19.5–48.9</td> </tr> <tr> <td>MECPP</td> <td>42.2–100.0</td> </tr> </tbody> </table>	Metabolite	NR	ΣDEHP		MEHP	3.8–11.2	MEHHP	31.8–80.8	MEOHP	19.5–48.9	MECPP	42.2–100.0	FRs for DEHP metabolites were ≥1, indicating no association or improved fecundability; none of the FRs were statistically significant (p>0.05) and there was no significant trend across tertiles of any metabolite (data shown graphically) DEHP metabolites were not associated with follicular phase length; higher MECPP levels were associated with longer luteal phase length (increase of ~0.5 days; p=0.02).						
Metabolite	NR																					
ΣDEHP																						
MEHP	3.8–11.2																					
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MECPP	42.2–100.0																					
Messerlian et al. 2016a; Hauser et al. 2015, Cohort (United States [Massachusetts])	215 women ages 18–46 years seeking infertility investigation; members of Environmental and Reproductive Health cohort, recruited between November 2004 and April 2012; excluding women with oophorectomies, unreadable ovary scans, and polycystic ovaries. Urine samples collected at study entry, twice during next treatment cycle, and at time of unstimulated AFC determination. All samples taken before AFC determination were pooled and geometric means of metabolite concentrations used in analysis.	Generalized linear models, adjusted for maternal age, BMI, and smoking status	Percent change in AFC comparing highest and lowest quartiles of urinary metabolite concentration <table border="1"> <thead> <tr> <th>Metabolite</th> <th>Concentration (µmol/L)</th> <th>Percent change</th> </tr> </thead> <tbody> <tr> <td>ΣDEHP</td> <td>0.10–0.46</td> <td>-14% (-23, -5)*</td> </tr> <tr> <td>MEHP</td> <td>1.6–6.7</td> <td>-13% (-22, -3)*</td> </tr> <tr> <td>MEHHP</td> <td>8.2–41.1</td> <td>-7% (-16, 4)</td> </tr> <tr> <td>MEOHP</td> <td>5.1–25.0</td> <td>-16% (-24, -6)*</td> </tr> <tr> <td>MECPP</td> <td>13.5–59.1</td> <td>-17% (-25, -7)*</td> </tr> </tbody> </table>	Metabolite	Concentration (µmol/L)	Percent change	ΣDEHP	0.10–0.46	-14% (-23, -5)*	MEHP	1.6–6.7	-13% (-22, -3)*	MEHHP	8.2–41.1	-7% (-16, 4)	MEOHP	5.1–25.0	-16% (-24, -6)*	MECPP	13.5–59.1	-17% (-25, -7)*	p for trend across quartiles >0.05 for all metabolites (p=0.06 for MEOHP). Hauser et al. (2015) observed statistically significant reductions in total and mature oocyte counts with higher urinary concentrations of DEHP metabolites in this cohort (n=256).
Metabolite	Concentration (µmol/L)	Percent change																				
ΣDEHP	0.10–0.46	-14% (-23, -5)*																				
MEHP	1.6–6.7	-13% (-22, -3)*																				
MEHHP	8.2–41.1	-7% (-16, 4)																				
MEOHP	5.1–25.0	-16% (-24, -6)*																				
MECPP	13.5–59.1	-17% (-25, -7)*																				

2. HEALTH EFFECTS

Table 2-11. Summary of Epidemiological Studies of DEHP Exposure and Female Reproductive Effects

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Sathyanara yana et al. 2017 Cross-Sectional (United States [California, Minnesota, New York, Washington])	591 pregnant women recruited from clinics in California, Minnesota, New York, and Washington between 2010 and 2012; members of Study for Future Families cohort. Urine and blood sample collected same day (59.5% at ≤12 weeks of gestation, 39.9% at >12–20 weeks of gestation, 0.5% at >20 weeks of gestation).	Linear regression, adjusted for study center, maternal age, maternal race/ethnicity, gestational age at serum draw, first-trimester BMI, and infant sex	Percent change in maternal hormone concentration per (unspecified) change in log-transformed SG-adjusted urinary metabolite concentration		
			ΣDEHP	15.73–39.70	Estrone 14.10 (-4.32, 36.08) Estradiol 11.02 (-4.02, 28.38) Total testosterone -5.57 (-15.08, 5.03) Free testosterone -9.18 (-18.57, -1.30)
			MEHP	1.38–4.35	Estrone 28.23 (9.85, 49.69)* Estradiol 24.97 (10.00, 41.97)* Total testosterone 0.00 (-9.01, 9.90) Free testosterone -3.46 (-12.38, 6.37)
			MEHHP	4.35–12.66	Estrone 12.62 (-3.88, 31.95) Estradiol 10.28 (-3.22, 25.69) Total testosterone -3.57 (-12.36, 6.10) Free testosterone -5.07 (-13.96, 4.74)
			MEOHP	3.22–8.46	Estrone 19.34 (1.39, 40.51)* Estradiol 14.71 (0.25, 31.22)* Total testosterone

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Table 2-11. Summary of Epidemiological Studies of DEHP Exposure and Female Reproductive Effects

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
				-4.79 (-13.72, 5.10) Free testosterone -6.59 (-15.61, 3.37)
			MECPP 5.89–15.71	Estrone 4.95 (-11.71, 24.74) Estradiol 3.18 (-10.55, 18.99) Total testosterone -7.87 (-16.98, 2.21) Free testosterone -12.30 (-21.17, -2.43)*
Johns et al. 2015, Cross-sectional (Puerto Rico)	106 pregnant women aged 18–40 years, members of Puerto Rico Test Site for Exploring Contamination Threats birth cohort, recruited at 14 weeks of gestation from prenatal clinics and hospitals.	Linear mixed models adjusted for age at enrollment, prepregnancy BMI, and urinary specific gravity	Percent change in serum estradiol, sex hormone binding globulin, and progesterone with IQR increase in urinary metabolite concentration (longitudinal analysis)	
	Urine and serum samples collected at 1 st and 3 rd prenatal visits (16–20 and 24–28 weeks of gestation).		ΣDEHP NR	Estradiol -0.56 (-9.17, 8.06) SBHG -4.11 (-9.83, 1.62) Ln-Progesterone 1.79 (-5.17, 9.39)
			MEHP Visit 1: 1.61–6.36; Visit 3: 1.69–6.73 (SG-adj)	NR
			MEHHP Visit 1: 6.14–19.9; Visit 3: 7.28–16.9	NR
			MEOHP Visit 1: 5.57–16.5; Visit 3: 6.22–14.8	NR
			MECPP Visit 1: 12.7–31.4; Visit 3: 13.4–29.3	NR
			Cross-sectional analyses by visit did not yield any statistically significant association or consistent pattern of change.	

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Table 2-11. Summary of Epidemiological Studies of DEHP Exposure and Female Reproductive Effects

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a		
Meeker and Ferguson 2014, Cross-sectional (United States)	697 women including 262 ages 20–40, 230 ages 40–60, and 205 ages 60–80; participants in NHANES 2011–2012. Urine and blood samples collected same day.	Linear regression adjusted for urinary Cr, age, poverty-income ratio, BMI, race/ethnicity, and session of sample collections	Percent change in serum total testosterone (ng/dL) with IQR increase in urinary metabolite concentration			
			ΣDEHP	Ages 20–<40	NR	-1.27 (-11.5, 10.1)
				Ages 40–<60		-20.1 (-30.9, -7.72)
				Ages 60–80		-2.24 (-21.0, 20.9)
			MEHP	Ages 20–<40	1.07, 3.57	2.54 (-10.9, 18.0)
				Ages 40–<60	0.90, 2.90	-13.4 (-27.0, 2.71)
				Ages 60–80	0.70, 1.94 (Cr-adj)	2.07 (-23.9, 36.9)
			MEHHP	Ages 20–<40	5.44, 14.6	-1.55 (-11.7, 9.77)
				Ages 40–<60	5.82, 15.4	-17.8 (-28.3, -5.72)
				Ages 60–80	5.27, 13.7	-2.46 (-19.6, 18.4)
			MEOHP	Ages 20–<40	3.62, 10.0	-0.19 (-10.5, 11.3)
				Ages 40–<60	3.73, 10.0	-21.8 (-31.6, -10.7)
				Ages 60–80	3.41, 8.38	-1.67 (-19.5, 20.2)
MECPP	Ages 20–<40	9.06, 21.7	-2.69 (-11.8, 7.31)			
	Ages 40–<60	10.4, 23.9	-18.7 (-29.2, -6.64)			
	Ages 60–80	9.98, 23.9	0.63 (-14.9, 19.0)			

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Table 2-11. Summary of Epidemiological Studies of DEHP Exposure and Female Reproductive Effects

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Sathyanara yana et al. 2014, Cross-sectional (United States [California, Minnesota, Missouri])	180 pregnant women recruited from prenatal clinics in California, Minnesota, and Missouri between September 1999 and August 2002; members of Study for Future Families cohort. Urine and blood sample collected same day (60% after 30 th week of gestation).	Linear regression adjusted for maternal age, gestational age at blood draw, urinary creatinine, study center, parity, and education	Association between serum hormone level and log-transformed urinary metabolite concentration in women with male fetuses (n=94)		
			ΣDEHP (MEHP, MEHHP, MEOHP)	5.53–21.05 μmol/L (all women)	Total testosterone -0.07 (-0.20, 0.06) Free testosterone -0.04 (-0.18, 0.10) Estradiol -0.06 (-0.17, 0.04)
			Association between log-transformed serum hormone level and log-transformed urinary metabolite concentration in women with female fetuses (n=86)		
			ΣDEHP (MEHP, MEHHP, MEOHP)	5.53–21.05 μmol/L (all women)	Total testosterone -0.15 (-0.26, -0.04)* Free testosterone -0.15 (-0.27, -0.03)* Estradiol -0.08 (-0.18, 0.01)

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

ΣDEHP = sum DEHP metabolites; AFC = antral follicle count; BMI = body mass index; CI = confidence interval; Cr = creatinine; Cr-adj = creatinine-adjusted; DEHP = di(2-ethylhexyl)phthalate; FR = fecundability ratio; IQR = interquartile range; LH = luteinizing hormone; 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; min = minimum; NHANES = National Health and Nutrition Examination Survey; NR = not reported; OR = odds ratio; SD = standard deviation; SG-adj = specific gravity adjusted

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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. Preterm birth as a categorical measure (<37 weeks of gestation) was evaluated in four epidemiological studies summarized in Table 2-12. Studies reporting associations between the odds of preterm birth and urinary DEHP metabolites include a nested case-control study (n=130 cases and 352 controls) by Ferguson et al. (2014a, 2014b) and a case-control study (n=30 cases and 30 controls) by Meeker et al. (2009b). Two cohort studies (Adibi et al. 2009; Shoaff et al. 2016; n=238 and 368, respectively) observed no association between exposure and preterm birth. In studies of gestational age as a continuous variable (Table 2-12), no clear relationship with urinary DEHP metabolite levels was seen. Of the six studies that evaluated gestational age, two (Adibi et al. 2009; Wolff et al. 2008) reported increased gestational age associated with increased urinary DEHP metabolite levels, and one (Whyatt et al. 2009) reported an association between decreasing gestational age and increasing metabolite levels. 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 (Yaghjian et al. 2016).

Only one study (Ferguson et al. 2014a) 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. (2014a; Table 2-12) observed an association between spontaneous preterm birth and the sum of DEHP metabolites in urine; 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. (2014a) 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

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Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a		
Preterm birth and gestational age						
Casas et al. 2016, Cohort (Spain)	Population-based birth cohort (INMA study) of 657 pregnant women recruited 2004–2006 during first prenatal visit.	Linear regression (considering these covariates: maternal education, smoking during pregnancy, parity, birth season, and urinary cotinine levels during pregnancy)	Estimated difference in gestational age (weeks) per doubling of log ₂ -transformed exposure levels			
			ΣDEHP	26.5–1,670 µg/g Cr (min–max)	β -0.13 (-1.72, 1.46)	
			MEHP	1.8–266.9 µg/g Cr	NA	
			MEHHP	5.3–503.4 µg/g Cr	NA	
			MEOHP	4.1–378.3 µg/g Cr	NA	
MECPP	7.7–718.9 µg/g Cr	NA				
Ferguson et al. 2014a, Case-control (United States [Massachusetts])	130 preterm births (<37 weeks) and 352 random controls selected from a prospective cohort of pregnant women recruited (2006-2008) early in pregnancy at Brigham and Women's Hospital in Boston.	Logistic regression (adjusting for specific gravity, maternal age at first visit, race/ethnicity, and education)	Risk of preterm birth per ln-unit increase in urinary phthalate metabolite level			
			ΣDEHP	20.2–63.2 µmol/mL (IQR; SG-adj)	OR 1.33 (1.04, 1.70)*	
			MEHP	5.51–18.1 (SG-adj)	OR 1.34 (1.07, 1.68)*	
			MEHHP	17.2–55.3	OR 1.03 (0.82, 1.30)	
			MEOHP	9.33–29.7	OR 1.16 (0.91, 1.47)	
	MECPP	20.6–73.8	OR 1.40 (1.13, 1.74)*			
	Gestational age determined by ultrasound and LMP recall. Spot urine samples collected during weeks 10, 18, and 26 of gestation.			Risk of spontaneous preterm birth per ln-unit increase in SG-adj urinary phthalate metabolite level		
				ΣDEHP	20.2–63.2 µmol/mL (IQR; SG-adj)	OR 1.63 (1.15, 2.31)*
				MEHP	5.51–18.1 (SG-adj)	OR 1.65 (1.20, 2.26)*
				MEHHP	17.2–55.3	OR 1.27 (0.91, 1.78)
MEOHP				9.33–29.7	OR 1.47 (1.04, 2.08)*	
MECPP	20.6–73.8	OR 1.56 (1.15, 2.13)*				

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Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Su et al. 2014, Cohort (Taiwan)	130 mother-infant pairs recruited and followed from November 2001–August 2009; TMICS.	Simple linear and binary logistic regression	Association between gestational age (weeks) and maternal urinary phthalate metabolite levels		
			ΣDEHP	42.28–60.83 µg/g Cr (95% CI)	β 0.001 (NR)
			MEHP	14.56–20.19 µg/g Cr	β 0.004 (NR)
			MEHHP	5.49–10.53 µg/g Cr	β 0.005 (NR)
			MEOHP	10.05–17.58 µg/g Cr	β 0.003 (NR)
MECPP	NA	NA			
Adibi et al. 2009, Cohort (United States [California, Iowa, Minnesota, Mississippi])	283 pregnant women in four states (Study for Future Families cohort; mean maternal age 30.2 years), recruited at the beginning of the 3 rd trimester from prenatal clinics from March 2000 to August 2004.	Linear and logistic regression (considering these covariates: creatinine, high blood pressure, and nongestational diabetes)	Change in log odds preterm birth per log-unit increase in urinary phthalate metabolite concentration		
			ΣDEHP	NA	NA
			MEHP	1.1–8.2	OR 0.5 (0.3, 0.9)*
			MEHHP	5.6–25.5	OR 0.5 (0.3, 0.9)*
			MEOHP	5.1–24.6	OR 0.4 (0.2, 0.9)*
			MECPP	NA	NA
			Gestational age determined by LMP recall and clinical estimate. Spot urine sample collected during 3 rd trimester.	Linear and logistic regression (considering these covariates: creatinine, geographic center, mother's educational level, job-related stress, nongestational diabetes, thyroid disorders, fibroids, and parity)	Change in gestational age (weeks) at delivery per log-unit increase in urinary phthalate metabolite concentration
ΣDEHP	NA	NA			
MEHP	1.1–8.2	β 0.16 (0.02, 0.3)*			
MEHHP	5.6–25.5	β 0.16 (0.01, 0.31)*			
MEOHP	5.1–24.6	β 0.19 (0.03, 0.35)*			
MECPP	NA	NA			

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Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Meeker et al. 2009b, Case-control (Mexico)	30 preterm births and 30 controls selected from a large Mexican birth cohort study; pregnant women were recruited during prenatal visits at one of four clinics of the Mexican Institute of Social Security in Mexico City between 2001 and 2003.	Multivariate logistic regression (considering these covariates: marital status, maternal education, infant sex, and gestational age at time of urine sample)	Odds of having Cr-adjusted urinary phthalate metabolite concentrations above the median in cases, compared with controls		
			ΣDEHP	Controls: 0.16–0.55 µg/g Cr (IQR); Cases: 0.28–0.45 µg/g Cr	OR 4.1 (1, 17.5)*
			MEHP	Controls: 1.7–7.4 µg/g Cr; Cases: 3.3–7.4 µg/g Cr	OR 3.2 (0.9, 11.3)
			MEHHP	Controls: 11.4–52.1 µg/g Cr; Cases: 24.1–41.5 µg/g Cr	OR 2.9 (0.8, 10.8)
			MEOHP	Controls: 9.5–42.1 µg/g Cr; Cases: 20.6–29.2 µg/g Cr	OR 3.2 (0.9, 11)
	Gestational age calculated from LMP recall.		MECPP	Controls: 27.3–98.6 µg/g Cr; Cases: 52.7–77.4 µg/g Cr	OR 2.9 (0.8, 11)
	Spot urine sample collected during 3 rd trimester.				
Wolff et al. 2008, Cohort (United States [New York])	404 mother-infant pairs enrolled prior to delivery at Mount Sinai Medical Center between March 1998 and March 2002 (Children's Environmental Health Study); mean±SD age: 24±6.2 years.	Multivariable linear regression (considering these covariates: Race, infant sex, ln-creatinine, smoking during pregnancy, maternal education, marital status, prepregnancy BMI, and restricted to observations with creatinine ≥20 mg/dL)	Association between gestational age (weeks) and ln-transformed maternal urinary phthalate metabolite concentration		
			ΣDEHP	0.13–0.5 µmol/L (IQR)	β 0.1 (-0.05, 0.24)
			MEHP	2.9–14	β 0.15 (0.02, 0.29)*
			MEHHP	9.5–39	β 0.06 (-0.07, 0.2)
			MEOHP	8.3–36	β 0.05 (-0.09, 0.2)
			MECPP	16–70	β 0.07 (-0.08, 0.21)

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Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Spontaneous abortion/pregnancy loss				
Jukic et al. 2016, Cohort (United States [North Carolina])	221 healthy women (median age 26 years, 96% white, 7% smokers) without fertility problems, recruited prior to conception between 1982 and 1986. Early pregnancy loss determined by decrease in urinary hCG level. Urine samples collected daily until clinical pregnancy demonstrated or 6 months after discontinuation of contraception. Samples analyzed 20 years later.	Unconditional logistic regression, considering these covariates: age, current smoking, alcohol intake, BMI, caffeine consumption, education, and season of conception	Adjusted odds of early pregnancy loss comparing highest tertile of exposure to lowest tertile <hr/> ΣDEHP MEHP 3.8–11.2 MEHHP 31.8–80.8 MEOHP 19.5–48.9 MECPP 42.2–100.0	Significantly (p<0.05) decreased odds of spontaneous abortion with higher MEOHP and sum DEHP metabolite levels (data shown graphically).
Messerlian et al. 2016b Cohort (United States [Massachusetts])	256 women with 303 conceived pregnancies (average age 34.9 years), recruited from women undergoing medically-assisted reproduction between 2004 and 2012 (Environment and Reproductive Health Study cohort).	Log-binomial regression models, adjusted for age, BMI, smoking status, and infertility diagnosis	Relative risk for biochemical pregnancy loss comparing highest quartile of exposure (SG-adj) with lowest quartile (p for trend) <hr/> ΣDEHP 0.10–0.40 μmol/L (IQR) MEHP 1.5–6.4 MEHHP 7.8–35.4 MEOHP 5.5–24.4	3.4 (0.97, 1.17) (p for trend = 0.04) <hr/> 2.8 (0.99, 8.1) (p for trend = 0.03) <hr/> 3.1 (0.91, 10.5) (p for trend = 0.03) <hr/> 5.2 (1.2, 21.9)* (p for trend = 0.006)

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Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
	Biochemical pregnancy loss defined as urinary hCG-confirmed pregnancy never visualized on ultrasound. Total pregnancy loss defined as any pregnancy loss <20 weeks of gestation (including biochemical pregnancy loss). Urine samples collected at study entry and up to 2 times per fertility treatment cycle.		MECPP 14.3–57.2	2.4 (0.78, 7.6) (p for trend = 0.07)
			Relative risk for total pregnancy loss (<20 weeks of gestation) between quartiles of SG-adjusted ln-transformed urinary metabolite	
			ΣDEHP 0.10–0.40 µmol/L (IQR)	1.6 (0.96, 2.7) (p for trend = 0.06)
			MEHP 1.5–6.4	1.6 (0.99, 2.7) (p for trend = 0.06)
			MEHHP 7.8–35.4	1.7 (1.0, 2.9)* (p for trend = 0.07)
			MEOHP 5.5–24.4	2.0 (1.1, 3.5)* (p for trend = 0.03)
			MECPP 14.3–57.2	1.4 (0.85, 2.4) (p for trend = 0.09)
Mu et al. 2015, Case-control (China)	132 cases of spontaneous abortion and 172 controls, aged 20–45 years, recruited prior to 20 weeks of gestation at time of ultrasound. Clinical pregnancy loss determined by transvaginal ultrasound. Urine sample collected on the 4 th day after ultrasound examination.	Logistic regression, adjusted for age, week of gestation, BMI, household income, smoking status, alcohol consumption, and occupation	Adjusted OR for clinical pregnancy loss comparing highest quartile of exposure with lowest quartile	
			ΣDEHP NA	NA
			MEHP Cases: 1.53–103 µg/g Cr (5 th –95 th percentiles) Controls: 1.27–20.8 µg/g Cr	OR 1.30 (0.66–2.55)

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Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Toft et al. 2012, Cohort (Denmark)	128 pregnant women with at least one urine sample, from prospective cohort of 430 couples aged 20–35 years recruited from trade unions in Denmark.	Logistic regression, adjusted for age, BMI, smoking, alcohol consumption, caffeine consumption, and exposure to the specific compound analyzed in the preconception cycle	Adjusted OR (95% CI) for early pregnancy loss comparing highest tertile of exposure to lowest tertile.		
			MEHP	<LOD–84 (pregnancy loss) (min–max) <LOD–64 (liveborn child)	OR 40.67 (4.48, 369.50)*
			MEHHP	9.5–207.1 (pregnancy loss) 3.6–215.3 (liveborn child)	OR 2.12 (0.67, 6.67)
			MEOHP	5.7–245.9 (pregnancy loss) 2.7–222.2 (liveborn child)	OR 2.73 (0.78, 9.54)
			Adjusted OR (95% CI) for clinical pregnancy loss comparing highest tertile of exposure to lowest tertile.		
			MEHP	<LOD–84 µg/L (pregnancy loss) (min–max) <LOD–64 µg/L (liveborn child)	OR 0.25 (0.05, 1.28)
			MEHHP	9.5–207.1 µg/L (pregnancy loss) 3.6–215.3 µg/L (liveborn child)	OR 0.90 (0.23, 3.61)
			MEOHP	5.7–245.9 µg/L (pregnancy loss) 2.7–222.2 µg/L (liveborn child)	OR 0.55 (0.13, 2.35)

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Table 2-12. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Pregnancy Outcomes

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Cantonwine et al. 2016, Case-control (United States [Massachusetts])	Nested case-control study (n=50 cases of preeclampsia and 431 pregnancies without preeclampsia) within prospective birth cohort at hospital in Boston. Preeclampsia defined by blood pressure (≥ 140 mm Hg systolic or ≥ 90 mm Hg diastolic) and positive urinary protein testing. Maternal urine samples collected at 9.7, 17.9, 26.0, and 35.1 weeks of pregnancy.	Cox proportional hazard (considering these covariates: specific gravity, maternal age, race, BMI, smoking during pregnancy, and infant sex)	Adjusted hazard ratio (95% CI) associated with IQR increase in concentration (average of three visits)		
			Σ DEHP	0.33–0.46 nmol/L (GMs on four visits)	HR = 1.79 (1.3, 2.46)*
			MEHP	9.8–12.7	HR = 1.4 (1.03, 1.89)*
			MEHHP	27.1–40.8	NR
			MEOHP	15.8–20.1	NR
			MECPP	38.5–41.8	NR

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

Σ DEHP = sum DEHP metabolites; BMI = body mass index; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; hCG = human chorionic gonadotropin; INMA = Infancia y Medio Ambiente; IQR = interquartile range; LMP = last menstrual period; 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; min = minimum; NA = not applicable; NR = not reported; OR = odds ratio; SD = standard deviation; SG-adj = specific gravity adjusted; TMICS = Taiwan Maternal and Infant Cohort Study

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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, was evaluated in three cohort studies and one case-control study that measured exposure using urinary metabolites of DEHP (Table 2-12). When evaluating early (or biochemical) pregnancy loss, one study observed decreased odds with increased urinary metabolite levels (Jukic et al. 2016), while two others reported increased risk of early pregnancy loss with an increase in urinary levels of one or more DEHP metabolites (Messerlian et al. 2016b; Toft et al. 2012). However, none of the three studies evaluating clinical pregnancy loss observed an association with exposure to DEHP (Messerlian et al. 2016b; Mu et al. 2015; Toft et al. 2012).

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 postimplantation loss in F0 dams after exposure to doses $\geq 1,040$ 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.

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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 a shorter-duration study, a complete absence of corpora lutea was observed in female B6C3F1 mice exposed to dietary doses of approximately 7,899 mg/kg/day DEHP; ovarian histology was not evaluated at lower doses in the study (Myers 1992a). 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).

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 postimplantation 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 ≥ 341 mg/kg/day, and complete litter losses at ≥ 500 mg/kg/day (Pocar et al. 2012; Price et al. 1988b; Schmidt et al. 2012; Shiota and Nishimura 1982; Shiota et al. 1980; Tyl et al. 1988). No changes in pregnancy outcomes were observed at ≤ 91 mg/kg/day. In 15 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 500 mg/kg/day in mice (Table 2-2).

Additional studies in rodents that did not evaluate reproductive performance show limited evidence of reproductive effects in non-pregnant female mice. A significant 25% decrease in serum estradiol levels was observed on GD 12.5 in dams exposed to 0.04 mg/kg/day via gavage from GD 0.5 to 19.5, compared with controls (Zhang et al. 2015); however, since no other dose levels were tested and no other reproductive endpoints were evaluated, the adversity of this finding is unclear. Therefore, reproductive effects from Zhang et al. (2015) were not included in the LSE table. In other intermediate-duration oral

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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, including 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 (Li et al. 2012b, 2016; Mu et al. 2015; Zhang et al. 2013b, 2014, 2015). Folliculogenesis effects appear to be mediated, in part, by DEHP or MEHP binding to PPARs and/or estrogen receptors (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. 2015).

Other than studies evaluating potential mechanisms for altered ovarian folliculogenesis, data on mechanisms of female reproductive toxicity are extremely limited. 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 ER α , progesterone receptor (PR), and E-cadherin in the mouse endometrium. The E-cadherin finding suggests that the MAPK and NF- κ B signaling pathways may be influenced by DEHP exposure. DEHP

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can also alter sterologogenesis 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 sterologogenesis from ¹⁴C-mevalonate in liver and adrenal minces (Bell 1980).

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.

2. HEALTH EFFECTS

Epidemiology Studies—Birth Size and Growth. Measures of birth size evaluated in epidemiological studies of DEHP include birth length, birth weight, and head circumference (Table 2-13). Only two of the seven selected studies that examined infant length, weight, or head circumference observed an association with DEHP metabolites in maternal or newborn urine (Sathyanarayana et al. 2016a; Zhao et al. 2014). 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. (2016a) reported increased birth weight in female infants, but not male infants, with increasing DEHP metabolite levels in maternal urine. Other studies did not observe an association between DEHP exposure and measures of birth size (Casas et al. 2016; Kim et al. 2016a; Shoaff et al. 2016; Su et al. 2014; Wolff et al. 2008).

Epidemiological studies evaluating the effects of prenatal exposure to DEHP and growth or obesity parameters in children have not shown consistent results, as shown in Table 2-14. Generally, the associations between maternal metabolite levels and BMI, waist circumference, and percent fat mass were negative, with higher DEHP exposures associated with lower BMI, waist circumference, and percent fat mass (Agay-Shay et al. 2015; Buckley et al. 2016a, 2016b; Maresca et al. 2016; Valvi et al. 2015). In contrast to the other studies, Harley et al. (2017) reported increased odds (Table 2-14) of being overweight or obese at 12 years of age when DEHP metabolite levels were doubled in maternal urine; however, sensitivity analysis indicated that maternal BMI influenced these results. 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 BMI z-score at 5–12 years or percent body fat at 9–12 years and maternal urinary DEHP levels. Kim et al. (2016a) also reported increased odds of higher growth (increase in BMI z-score more than the 50th 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.

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).

2. HEALTH EFFECTS

Table 2-13. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β [95% CI]) unless otherwise specified ^a	
Casas et al. 2016, Cohort (Spain)	Population-based birth cohort (INMA study) of 657 pregnant women recruited 2004–2006 during first prenatal visit.	Linear regression adjusted for maternal education, smoking during pregnancy, parity, birth season, and urinary cotinine levels during pregnancy	Estimated difference per doubling of log ₂ -transformed urinary metabolite concentrations		
			Σ DEHP	26.5–1,670 $\mu\text{g/g Cr}$ (min–max)	Birth length: Difference 0.38 (-1.59, 2.35) Birth weight: Difference 15.56 (-28.75, 59.87) Head circumference: Difference 0.16 (-1.15, 1.47)
			MEHP	1.8–266.9 $\mu\text{g/g Cr}$	NR
			MEHHP	5.3–503.4 $\mu\text{g/g Cr}$	NR
			MEOHP	4.1–378.3 $\mu\text{g/g Cr}$	NR
Kim et al. 2016a, Cohort (Korea)	128 infants (65 boys and 63 girls) from birth cohort (Children's Health and Environmental Chemicals in Korea cohort); pregnant women recruited from five hospitals in four cities just prior to delivery of singleton birth. Newborns' first urine samples collected within 2 days of birth; infants' weights and heights recorded at birth, and obtained through telephone interview with mothers at 3 months of age.	Linear regression adjusted for maternal age, maternal BMI, gestational period, caesarean section, delivery experience, and urinary Cr	Association between birth size metrics and log-transformed urinary metabolite concentrations in newborn urine in boys		
			Birth length		
			Σ DEHP	NR	0.050 (0.017, 0.082)*
			MEHHP	3.21–11.87	0.048(0.015, 0.080)*
			MEOHP	1.51–6.50)	0.052 (0.019, 0.085)*
			Birth weight		
			Σ DEHP	NR	-0.001 (-0.050, 0.048)
			MEHHP	3.21–11.87	-0.003 (-0.052, 0.045)
			MEOHP	1.51–6.50	0.003 (-0.046, 0.052)
			Head circumference		
			Σ DEHP	NR	-0.001 (-0.050, 0.048)
MEHHP	3.21–11.87	-0.0004 (-0.021, 0.020)			
MEOHP	1.51–6.50	0.003 (-0.018, 0.024)			
No significant associations were seen in girls or analysis of combined genders					

2. HEALTH EFFECTS

Table 2-13. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)		Regression coefficient (β [95% CI]) unless otherwise specified ^a
Sathyanarayana et al. 2016a, Cohort (United States [California, Minnesota, New York, Washington])	753 mother-infant pairs recruited from one of four study centers (University of California, San Francisco, University of Minnesota, University of Rochester Medical Center, Seattle Children's Hospital) between 2010 and 2012 (TIDES study).	Linear regression adjusted for race, smoking during pregnancy, study center, parity, income, and gestational age at birth	Change in birth weight in per log-unit increase in SG-adjusted urinary phthalate metabolite concentration		
			Σ DEHP	NR	Males: -0.03 (-0.16, 0.10) Females: 0.16 (0.03, 0.29)*
			MEHP	1.37–4.35	Males: -0.02 (-0.14, 0.10) Females: 0.13 (0.01, 0.24)*
			MEHHP	4.35–12.77	Males: -0.03 (-0.15, 0.08) Females: 0.15 (0.03, 0.27)*
			MEOHP	3.13–8.70	Males: -0.05 (-0.17, 0.07) Females: 0.13 (0.00, 0.25)*
MECPP	5.90–15.95	Males: -0.01 (-0.14, 0.12) Females: 0.14 (0.02, 0.26)*			
Shoaff et al. 2016, Cohort (United States [Ohio])	368 mother-infant pairs recruited from one of seven prenatal care clinics in Cincinnati, Ohio between 2003 and 2006 (HOME study).	Linear regression adjusted for maternal race, age, income, education, marital status, insurance, parity, food security, prenatal vitamin use, fish consumption, fruit/vegetable consumption, BMI, BDI score, and serum cotinine level	Change in birth weight per 10-fold increase in urinary phthalate metabolite concentration		Presented graphically; non-significant for sum DEHP metabolites
			Σ DEHP	16 weeks: 0.14–0.72 nmol/mL 26 weeks: 0.10–0.52 nmol/mL	
			MEHP	16 weeks: 1.30–13.00 26 weeks: 1.60–10.40	
			MEHHP	16 weeks: 11.60–61.50 26 weeks: 8.30–46.60	
			MEOHP	16 weeks: 9.10–45.80 26 weeks: 7.00–37.50	
MECPP	16 weeks: 15.80–89.30 26 weeks: 12.70–63.50				

2. HEALTH EFFECTS

Table 2-13. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β [95% CI]) unless otherwise specified ^a	
Su et al. 2014, Cohort (Taiwan)	130 mother-infant pairs recruited and followed from November 2001 to August 2009; TMICS.	Simple linear and binary logistic regression adjusted for gestational age (used to calculate z-score)	Association with maternal urinary metabolite concentrations		
			Birth length		
			Σ DEHP	42.28–60.83 $\mu\text{g/g Cr}$ (95% CI)	0.001 (NR)
			MEHP	14.56–20.19 $\mu\text{g/g Cr}$	0.002 (NR)
			MEHHP	5.49–10.53 $\mu\text{g/g Cr}$	0.002 (NR)
			MEOHP	10.05–17.58 $\mu\text{g/g Cr}$	0.001 (NR)
			Birth weight		
			Σ DEHP	42.28–60.83 $\mu\text{g/g Cr}$ (95% CI)	0.001 (NR)
			MEHP	14.56–20.19 $\mu\text{g/g Cr}$	0.001 (NR)
			MEHHP	5.49–10.53 $\mu\text{g/g Cr}$	0.002 (NR)
			MEOHP	10.05–17.58 $\mu\text{g/g Cr}$	0.001 (NR)
			Head circumference		
			Sum	42.28–60.83 $\mu\text{g/g Cr}$ (95% CI)	0.001 (NR)
			MEHP	14.56–20.19 $\mu\text{g/g Cr}$	0.002 (NR)
			MEHHP	5.49–10.53 $\mu\text{g/g Cr}$	0.003 (NR)
			MEOHP	10.05–17.58 $\mu\text{g/g Cr}$	0.002 (NR)

2. HEALTH EFFECTS

Table 2-13. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β [95% CI]) unless otherwise specified ^a	
Wolff et al. 2008, Cohort (United States [New York])	404 mother-infant pairs enrolled prior to delivery at Mount Sinai Medical Center between March 1998 and March 2002 (Children's Environmental Health Study); mean \pm SD age: 24 \pm 6.2 years.	Multivariable linear regression adjusted for race, infant sex, gestational age, In-creatinine, smoking during pregnancy, maternal education, marital status, prepregnancy BMI, and restricted to observations with creatinine \geq 20 mg/dL	Association with ln-transformed maternal urinary metabolite concentrations		
			Birth length		
			Σ DEHP	0.13–0.5 μ mol/L	0.07 (-0.13, 0.27)
			MEHP	2.9–14	0.01 (-0.18, 0.19)
			MEHHP	9.5–39	0.08 (-0.10, 0.27)
			MEOHP	8.3–36	0.07 (-0.12, 0.27)
			MECPP	16–70	0.04 (-0.16, 0.24)
			Birth weight		
			Σ DEHP	0.13–0.5 μ mol/L	10 (-29, 49)
			MEHP	2.9–14	4.9 (-28, 38)
			MEHHP	9.5–39	6.6 (-27, 40)
			MEOHP	8.3–36	5.1 (-29, 40)
			MECPP	16–70	4.2 (-31, 40)
			Head circumference		
			Σ DEHP	0.13–0.5 μ mol/L	0.00 (-0.14, 0.14)
MEHP	2.9–14	0.01 (-0.11, 0.14)			
MEHHP	9.5–39	0.00 (-0.13, 0.13)			
MEOHP	8.3–36	0.01 (-0.12, 0.14)			
MECPP	16–70	0.01 (-0.13, 0.14)			

2. HEALTH EFFECTS

Table 2-13. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Birth Size

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β [95% CI]) unless otherwise specified ^a	
Zhao et al. 2014, Case-control (China)	42 IUGR infants and 84 controls (2 per case, matched for maternal age); mother-infant pairs were recruited during 3 rd trimester sonogram examination at the Second Affiliated Hospital of Wenzhou Medical College between March 2012 and January 2013.	Logistic regression adjusted for maternal age, gestational age at delivery, maternal education, prepregnancy BMI, passive smoking, and other urinary phthalate metabolite concentrations	Risk of IUGR in the highest tertile of urinary phthalate metabolite concentration, compared with lowest (cases and controls combined)		
			Σ DEHP	All: 13.6–46.3 Cases: 16.4–54.5; Controls: 9.3–41.5	No significant ($p < 0.05$) association between birth length and urinary levels of metabolites after adjustment for covariates (data presented graphically)
			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	Significant ($p < 0.05$) association between lower birth weight and increasing urinary levels of MEHHP and MEOHP (data presented graphically)
MEOHP	All: 1.7–9.7 Cases: 2.4–15.0 Controls: 1.4–6.4	Significantly increased adjusted OR for IUGR and MEHHP (data presented graphically)			

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

Σ DEHP = sum DEHP metabolites; BDI = Beck Depression Inventory; BMI = body mass index; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)-phthalate; HOME = Health Outcomes and Measures of the Environment; INMA = Infancia y Medio Ambiente; IQR = interquartile range; IUGR = intrauterine growth retardation; 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; min = minimum; NR = not reported; OR = odds ratio; SD = standard deviation; TIDES = The Infant Development and Environment Study; TMICS = Taiwan Maternal and Infant Cohort Study

2. HEALTH EFFECTS

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and BMI, Waist Circumference, and Fat Mass

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Harley et al. 2017, Cohort (United States [California])	345 children from the CHAMACOS birth cohort in California enrolled between 1999 and 2000. Maternal urine samples collected at mean gestation weeks 14.0 and 26.9; children's weight, height, and waist circumference were recorded at 5, 7, 9, 10.5, and 12 years.	Logistic and linear regression adjusted for maternal age, education, marital status; mother's years of residence in the United States and family income at the time of pregnancy; smoking during pregnancy; and repeated measures of family food insecurity and child's fast food consumption at each time point	Odds of being overweight or obese (BMI $\geq 85^{\text{th}}$ percentile) at 5–12 years per doubling of log-transformed maternal urinary metabolite concentration		
			Σ DEHP	0.1–0.3 $\mu\text{mol/L}$	5 years: OR 1.1 (0.9, 1.4) 7, 9, 10.5 years: OR 1.2 (1.0, 1.5) 12 years: OR 1.3 (1.0, 1.6)*
			MEHP	2.1–7.0	NR
			MEHHP	8.6–27.8	NR
			MEOHP	6.6–20.8	NR
			MECCP	15.7–43.1	NR
			Change in BMI z-score at ages 5–12 years per each doubling of log-transformed maternal urinary metabolite		
			Σ DEHP	0.1–0.3 $\mu\text{mol/L}$	5 years: β 0.05 (-0.05, 0.16) 7 years: β 0.08 (-0.02, 0.18) 9 years: β 0.09 (-0.01, 0.20) 10.5 years: β 0.09 (-0.02, 0.19) 12 years: β 0.08 (-0.03, 0.19)
			Change in waist circumference z-score at ages 5–12 years per each doubling of log-transformed maternal urinary metabolite		
			Σ DEHP	0.1–0.3 $\mu\text{mol/L}$	5 years: β 0.14 (0.05, 0.23)* 7 years: β 0.00 (-0.08, 0.09) 9 years: β 0.00 (-0.09, 0.09) 10.5 years: β 0.10 (0.00, 0.19) 12 years: β 0.09 (-0.01, 0.20)
			Change in percent body fat at ages 9–12 years per each doubling of log-transformed maternal urinary metabolite		
			Σ DEHP	0.1–0.3 $\mu\text{mol/L}$	9 years: β 1.0 (-0.2, 2.2) 10.5 years: β 1.0 (-0.2, 2.2) 12 years: β 1.1 (-0.2, 2.4)

2. HEALTH EFFECTS

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and BMI, Waist Circumference, and Fat Mass

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Buckley et al. 2016a, Cohort (United States [New York and Ohio])	707 children from three birth cohorts (Mount Sinai Children's Environmental Health and Disease Prevention Research Center in New York, The CCCEH in New York, and Health Outcomes and Measures of the Environment in Ohio) enrolled between 1998 and 2006. Maternal urine samples collected at mean gestation weeks between 27 and 34; children's weights and heights recorded at ages between 4 and 7 years.	Linear mixed-effects regression adjusted for cohort; maternal race/ethnicity, age at delivery, education, work status during pregnancy, prepregnancy BMI, height, gestational weight gain, smoking during pregnancy, natural log Cr, calendar date of urine collection, parity, breast feeding, and child's sex and months of age at followup	Change in BMI z-score at ages 4–7 years per 1 SD increase in ln-transformed maternal urinary metabolite concentration (data pooled across cohorts)		
			Σ DEHP		
				0.128–0.562 μ mol/L	β -0.04 (-0.15, 0.06)
			MEHP	2.00–11.9	NR
			MEHHP	9.20–45.1	NR
			MEOHP	8.00–37.5	NR
		MECPP	16.1–74.4	NR	
Analyses by child's sex, race/ethnicity, and cohort also did not identify any significant association with DEHP metabolites.					
Maresca et al. (2016) evaluated the same outcome in a subset of this cohort (members of the CCCEH cohort); no significant association was observed.					
Buckley et al. 2016b, Cohort (United States [New York])	180 children (82 girls and 98 boys) from birth cohort (Mount Sinai Children's Environmental Health Study); mothers recruited between 1998 and 2002 from Mount Sinai Hospital and two private practices. Maternal urine samples collected between 25 and 40 weeks of gestation. Children's fat mass was measured at ages 4 and 9 years.	Linear mixed-effects regression adjusted for prepregnancy BMI, gestational weight gain, maternal smoking during pregnancy, and breastfeeding	Change in percent fat mass in children 4–9 years old per 1 SD increase in ln-transformed maternal urinary metabolite concentration		
			Σ DEHP		
				125–530 nmol/L	β -0.89 (-2.24, 0.47)
			MEHP	3.00–14.2	NR
			MEHHP	8.80–41.3	NR
			MEOHP	8.20–38.3	NR
		MECPP	15.1–72.7	NR	
Analyses stratified by child's sex did not result in statistically significant effect estimates or change the direction of change.					

2. HEALTH EFFECTS

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and BMI, Waist Circumference, and Fat Mass

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified) Effect estimate (95% CI) ^a		
Kim et al. 2016a, Cohort (Korea)	128 infants (65 boys and 63 girls) from birth cohort (Children's Health and Environmental Chemicals in Korea cohort); pregnant women recruited from five hospitals in four cities just prior to delivery of singleton birth. Newborns' first urine samples collected within 2 days of birth; infants' weights and heights recorded at birth, and obtained through telephone interview with mothers at 3 months of age.	Logistic regression adjusted for sex, birth weight, birth length, head circumference at birth, ponderal index (ratio of height to weight) at birth and 3 months; and leptin, total cholesterol, and triglyceride in cord blood and at 3 months	OR for BMI z-score increase >50 th percentile from birth to 3 months per log-unit increase in newborn urinary metabolite concentration		
			ΣDEHP	NR	OR 4.35 (1.2, 15.72)*
			MEHHP	3.21–11.87	OR 4.43 (1.22, 16.04)*
			MEOHP	1.51–6.50	OR 3.91 (1.12, 13.65)*
			Association between triglyceride or cholesterol in cord blood and log-transformed newborn urinary metabolite concentration		
			ΣDEHP	NR	Total cholesterol β -0.019 (-0.103, 0.065) Triglyceride β 0.144 (0.020, 0.267)*
			MEHHP	3.21–11.87	Total cholesterol β -0.021 (-0.104, 0.062) Triglyceride β 0.146 (0.024, 0.267)*
			MEOHP	1.51–6.50	Total cholesterol β -0.014 (-0.098, 0.070) Triglyceride β 0.132 (0.009, 0.256)*
			Agay-Shay et al. 2015; Valvi et al. 2015, Cohort (Spain)		
MEHP	1.8–266.9 µg/g Cr (min–max)	β -0.03 (-0.32, 0.27)			
MEHHP	5.3–503.4 µg/g Cr (β -0.09 (-0.39, 0.21)			
MEOHP	4.1–378.3 µg/g Cr	β -0.14 (-0.44, 0.16)			
MECPP	7.7–718.9 µg/g Cr	β -0.26 (-0.55, 0.04)			
			Inclusion of imputed values did not significantly alter the results.		

2. HEALTH EFFECTS

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and BMI, Waist Circumference, and Fat Mass

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
<p>Children from birth cohort (INMA or Environment and Childhood cohort); mothers were >16 years old with singleton pregnancy, enrolled at ultrasound during first trimester between 2004 and 2006. Maternal urine samples collected during 1st and 3rd trimesters, at mean gestational weeks 13 and 34 weeks, respectively. Children's heights and weights measured at birth and ages 6 months and 1, 4, and 7 years.</p> <p>Agay-Shay et al. (2015) included 470 children with followup data from 7 years of age.</p> <p>Valvi et al. (2015) evaluated subset of 391 children with complete data, including measurements at 1, 4, and 7 years.</p>	<p>Agay-Shay et al. (2015): Linear regression adjusted for child's sex, gestational age, birth weight, exact age at the time that the outcome was measured (months), maternal country of origin, maternal age at delivery, maternal prepregnancy BMI, maternal weight gain during pregnancy, maternal social class, breastfeeding duration, and maternal smoking during pregnancy</p> <p>Valvi et al. (2015): Generalized estimating equations adjusted for child's exact age at examination and maternal characteristics (country of origin, age at delivery, parity, education, social class, prepregnancy BMI, and smoking in pregnancy)</p>	Association between BMI z-score and log-transformed, Cr-adjusted average maternal urinary metabolite concentration (Valvi et al. 2015) (urine concentrations shown for boys and girls combined)		
		ΣDEHP	64.9–139 µg/g Cr (as MEHP)	Boys: β -0.32 (-0.64, -0.02)* Girls: β 0.21 (-0.11, 0.53)
		MEHP	7.3–17.2 µg/g Cr	NR
		MEHHP	17.9–41.5 µg/g Cr	NR
		MEOHP	14.3–30.3 µg/g Cr	NR
		MECPP	31.0–61.2 µg/g Cr	NR

2. HEALTH EFFECTS

Table 2-14. Summary of Epidemiological Studies of Prenatal DEHP Exposure and BMI, Waist Circumference, and Fat Mass

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Maresca et al. 2016, Cohort (United States [New York])	424 children from birth cohort (Columbia Center for Children's Environmental Health cohort); pregnant African-American or Dominican women between 18 and 35 years of age recruited from two New York city hospitals between 1998 and 2006. Maternal urine samples collected during 3 rd trimester. Children's anthropometric parameters measured at age 7.	Linear regression adjusted for maternal race/ethnicity, receipt of public assistance during pregnancy, prepregnancy obesity status, child birth weight, child age in months at time of followup, and urine specific gravity	Change in waist circumference at age 7 per unit increase in ln-transformed maternal urinary metabolites		
			ΣDEHP	292.89 (3.24) nmol/L (GM [GSD])	Boys: β -0.65 (-2.16, 0.87) Girls: β -0.13 (-1.37, 1.12)
			MEHP	4.91 (4.21)	NR
			MEHHP	22.03 (3.56)	NR
			MEOHP	18.30 (3.48)	NR
			MECPP	39.04 (3.08)	NR
			Change in percent body fat at age 7 per unit increase in ln-transformed maternal urinary metabolites		
			ΣDEHP	292.89 (3.24) nmol/L (GM [GSD])	Boys: β -0.39 (-1.57, 0.79) Girls: β -0.13 (-1.09, 0.84)
			MEHP	4.91 (4.21)	NR
			MEHHP	22.03 (3.56)	NR
MEOHP	18.30 (3.48)	NR			
MECPP	39.04 (3.08)	NR			

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

ΣDEHP = sum DEHP metabolites; BMI = body mass index; CCCEH = Columbia Center for Children's Environmental Health; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; GSD = geometric standard deviation; INMA = Infancia y Medio Ambiente; IQR = interquartile range; 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; min = minimum; NR = not reported; OR = odds ratio; SD = standard deviation

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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; Price et al. 1988b; Schilling et al. 1999, 2001; Tanaka 2002; Tomita et al. 1982a; Yagi et al. 1980). Several studies also 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. 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). 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 (Price et al. 1988b).

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 $\geq 10\%$ 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.

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(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). 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 lactational exposure to maternal doses up to 500 mg/kg/day (Carbone et al. 2010, 2012; Dalsenter et al. 2006; Schilling et al. 1999, 2001); however, Christiansen et al. (2010) reported decreased offspring weights at doses ≥ 300 mg/kg/day. 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 at ≥ 191 mg/kg/day, but not ≤ 100 mg/kg/day (Maranghi et al. 2010; Price et al. 1988b; Shiota et al. 1980; Shiota and Nishimura 1982; Tyl et al. 1988). Similarly, a 1-generation study reported a lack of body weight effects in offspring at maternal doses up to 180.77 mg/kg/day (Tanaka 2002). However, 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). In contrast, another 1-generation study reported a significant *increase* in F1 offspring body weight and visceral adipose tissue at doses ≥ 0.05 mg/kg/day (Schmidt et al. 2012). 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). 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 500 mg/kg/day for 15 days post-weaning (Vo et al. 2009b). Unspecified body weight decreases and increased mortality were observed in neonatal and weanling rats exposed to $\geq 1,000$ mg/kg/day DEHP via

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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 $\geq 5,700$ and $6,200$ mg/kg/day, respectively, and male and female B6C3F1 mice at $\geq 4,900$ 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 pregnancy loss, preterm birth, low birth weight, and IUGR, including alteration of ovarian steroidogenesis, thyroid dysfunction, placental alterations, and intrauterine inflammation (Marie et al. 2015).

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 PPAR γ activation, perturbation of PPAR γ -induced regulators of adipogenesis and lipogenesis, and increased adipocyte differentiation (Hao et al. 2012). Perturbation of PPAR γ -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

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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).

Transient 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). Transient 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.

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 at PND 21 or 63 in offspring born to Sprague-Dawley rat dams exposed to DEHP at doses up to 400 mg/kg/day from GD 6 to PND 20 (Kobayashi et al. 2006). 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).

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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. Histological examination showed decreased glomerular size, glomerular swelling, and reduction in Bowman's capsule size in both exposure groups from PND 0 to PNW 33.

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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. Significant changes observed in offspring kidney weights included decreased absolute weight in high-dose females at PNW 15, increased absolute weight in high-dose males at PNW 21, increased relative weight in high-dose pups at PNDs 0 and 3, increased relative weight in low-dose females at PNW 15, and increased relative weight in high-dose males at PNWs 15 and 21 (Wei et al. 2012).

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 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). 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). Increased relative kidney weights were observed in neonatal and weanling rats exposed to $\geq 1,000$ mg/kg/day DEHP via gavage for 5 days (Dostal et al. 1987). Measures of renal function and kidney histology were not assessed in these studies.

Epidemiology Studies—Neurodevelopment. Many epidemiological studies assessed neurodevelopmental 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 are birth cohort studies that evaluated exposure using maternal urine samples.

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In a study using the neonatal intensive care unit (NICU) Network Neurobehavioral Scale (NNS) 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 ($\beta = 0.216$, 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 NNS (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 eight studies of birth cohorts evaluating 110–460 children (Table 2-15). 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. Most studies administered the tests at one point in time, although Tellez-Rojo et al. (2013) and Huang et al. (2015) conducted longitudinal analyses, using repeated test scores in the same children. Two studies suggested associations between poorer performance on the mental development index at 6 months (Kim et al. 2011) and 2–3 years of age (Tellez-Rojo et al. 2013) and prenatal DEHP exposure. The affected sex differed between the studies with Kim et al. (2011) reporting an association for male infants and Tellez-Rojo et al. (2013) observing an association only in female infants. Two studies (Kim et al. 2011; Polanska et al. 2014) reported associations between prenatal DEHP exposure and psychomotor development in young children (6 months and 2 years, respectively). Other studies (Doherty et al. 2017; Factor-Litvak et al. 2014; Gascon et al. 2015b; Huang et al. 2015; Whyatt et al. 2012) did not observe associations between cognitive, mental, or psychomotor development and maternal urinary metabolites of DEHP (Table 2-15). 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.

Studies examining potential relationships between DEHP exposure and autism spectrum disorders 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 considered useful for hazard identification. Miodovnik et al. (2011) and Braun et al. (2014) examined autism-related behavior in two U.S. birth

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Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference and study type	Population	Method of analysis	Metabolite concentration in urine (IQR in ng/mL unless otherwise specified)	Results ^a
Infant neurological status				
Yolton et al. 2011, Cohort (United States [Ohio])	350 infants (163 boys, 187 girls); members of birth cohort (HOME); mothers at ~16 weeks of gestation recruited from nine obstetrical clinics between 2003 and 2006. Maternal urine collection at 16 and 26 weeks of gestation. NNNS at 5 weeks of age.	Linear regression adjusted for urinary creatinine, infant age at exam and weight change since birth, and maternal income	Σ DEHP 16 weeks: 311 (269–360) 26 weeks: 245 (213–281) (GM [95% CI], in nmol/L)	NNNS at 5 weeks: ↑ frequency of nonoptimal reflexes in male infants with ↑ Σ DEHP metabolites in 26-week (but not 16-week) maternal urine β 0.216* 95% CIs not reported
			MEHP 16 weeks: 4.9 (4.2–5.7) 26 weeks: 4.2 (3.7–4.9)	No significant association in female infants, or on other subscales of the NNNS in males or females.
			MEHHP 16 weeks: 26.9 (23.0–31.3) 26 weeks: 20.4 (17.6–23.5)	
			MEOHP 16 weeks: 19.9 (17.1–23.2) 26 weeks: 16.5 (14.3–19.1)	
			MECPP 16 weeks: 38.0 (33.0–43.7) 26 weeks: 29.9 (26.1–34.2)	

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Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference and study type	Population	Method of analysis	Metabolite concentration in urine (IQR in ng/mL unless otherwise specified)	Results ^a
Mental, psychomotor, and cognitive development (by age at evaluation, youngest to oldest)				
Kim et al. 2011, Cohort (Korea)	460 children (235 boys and 225 girls) aged 6 months; members of birth cohort (Mothers and Children's Environmental Health); mothers in their 1 st trimester were recruited at obstetric clinics in three cities between 2006 and 2009. Single maternal urine sample collected between 36 and 42 weeks of gestation; BSID-II (MDI and PDI) administered to infants at 6 months of age.	Linear regression adjusted for infant birth weight, infant sex, maternal age, maternal education level, family income, breastfeeding status, and residential area	MEHHP 4.3–21.4 MEOHP 3.8–17.1	BSID II at 6 months: ↓ MDI scores in male infants with ↑ MEHHP and MEOHP in maternal urine β -1.46* and -1.57*, respectively ↓ PDI scores in male infants with ↑ MEHHP and MEOHP in maternal urine β -2.36* and -2.05*, respectively 95% CIs not reported No significant association in female infants; in analyses grouping across sex, significant associations between MDI and PDI and DEHP metabolites were also seen. Significant association also seen in subgroup analysis that controlled for maternal intelligence score.
Gascon et al. 2015b, Cohort (Spain)	367 children (187 boys, 178 girls); members of birth cohort (INMA or Environment and Childhood); mothers recruited at 1 st trimester prenatal visit at hospital or health center between 2004 and 2006. Maternal urine samples collected at 12 and 32 weeks of gestation and results averaged. BSID (MDI and PDI) administered to infants at 1 year of age, and MSCA administered at age 4.	Linear regression adjusted for sex, maternal age and education, maternal smoking during pregnancy, birth season, breastfeeding, maternal country of origin, number of siblings, and child's age (BSID not adjusted for child's age)	Σ DEHP 68–146 μ g/g Cr MEHP 7–17 μ g/g Cr MEHHP 18–41 μ g/g Cr MEOHP 14–30 μ g/g Cr MECPP 27–59 μ g/g Cr	BSID at 1 year: No significant association between MDI or PDI scores and Σ DEHP metabolites in maternal urine; no sex-specific associations observed. MSCA at 4 years: No significant association between any test subscore and Σ DEHP metabolites in maternal urine; no sex-specific associations observed.

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Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference and study type	Population	Method of analysis	Metabolite concentration in urine (IQR in ng/mL unless otherwise specified)	Results ^a
Doherty et al. 2017, Cohort (United States [New York])	250 children (134 boys, 116 girls); from a cohort of 404 pregnant women; mothers recruited at first prenatal visit (<26 weeks) at hospital or health center 1998-2002. Single maternal urine sample (n=258) collected at 25-40 weeks of gestation. BSID (MDI and PDI) administered at approximately 24 months of age.	Linear regression adjusted for urinary creatinine, pre-pregnancy BMI, maternal race, maternal education, HOME Score, duration of breastfeeding, maternal age, child age at testing, child's sex (male/female), and maternal marital status	Σ DEHP 0.28 (3.7) μ mol/L (GM [SE]) MEHP 6.2 (3.8) MEHHP 20 (4.0) MEOHP 18 (3.9) MECPP 35 (3.7)	BSID at 24 months: No significant association between MDI or PDI scores and Σ DEHP metabolites in maternal urine; no sex-specific associations were observed.
Polanska et al. 2014, Cohort (Poland)	165 children (72 boys, 93 girls); members of birth cohort (Polish Mother and Child Cohort); mothers recruited during first trimester of pregnancy at maternity units or clinics (time of recruitment not reported). Single maternal urine sample collected during 3 rd trimester (30–34 weeks of gestation); child urine sample collected at 24 months of age. BSID-III administered to infants at 24 months of age.	Linear regression adjusted for examiner, parental age, parental education, child gender, pre- and postnatal ETS exposure, cognitive development, marital status, and child nursery attendance	Σ DEHP 0.0004–1.5 μ mol/g Cr (min–max) MEHP 0.02–4.3 μ g/g Cr MEHHP 0.02–431 μ g/g Cr MEOHP 0.04–140 μ g/g Cr	BSID-III at 2 years: No significant association with cognitive or language scores after adjustment for covariates. ↓ motor scores with ↑ log-transformed Cr-adjusted MEHHP, MEOHP, and Σ DEHP metabolites in maternal urine β -1.2*, -1.8*, and -2.2*, respectively 95% CIs not reported Analyses using child urine samples were not considered, as outcome was measured concurrently.

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Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference and study type	Population	Method of analysis	Metabolite concentration in urine (IQR in ng/mL unless otherwise specified)	Results ^a
Tellez-Rojo et al. 2013, Cohort (Mexico)	135 children (64 boys, 71 girls); members of birth cohort (Early Life Exposure in Mexico to Environmental Toxicants); mothers recruited during 1 st trimester (no further details). Single maternal urine sample collected during 3 rd trimester. BSID-II administered to children at 24, 30, and 36 months of age (results combined in analysis).	Linear regression for longitudinal data adjusted for birth weight, breastfeeding practices, Z-scores for weight-for-age, child's current age, mother's age, mother's educational level, and laboratory where urine analyzed	Σ DEHP 0.35 (0.30, 0.40) nmol/mL (GM [95% CI], SG-adj) MEHP 6.56 (5.72, 7.53) MEHHP 22.08 (18.77, 25.96) MEOHP 14.23 (12.05, 16.80) MECPP 39.65 (34.32, 45.81)	BSID-II between 2 and 3 years: ↓ MDI scores in girls* with ↑ ln-transformed MEHP, MEHHP, MEOHP, MECPP, and Σ DEHP metabolites in maternal urine β -2.11*, -1.89*, -1.80*, -2.52*, and -3.41*, respectively 95% CIs not reported No significant association with MDI in boys or combined analyses. No significant association with PDI in combined or sex-stratified analyses.
Whyatt et al. 2012, Cohort (United States [New York])	319 children (151 boys, 168 girls); members of birth cohort (CCCEH); black or Dominican mothers recruited prior to 20 th week of pregnancy (no further details). Single maternal urine sample collected during 3 rd trimester (mean 33 weeks of gestation). BSID-II administered to infants between 27 and 42 months of age (mean 36.4 months).	Linear regression adjusted for child sex, race/ethnicity, quality of proximal care-taking environment, gestational age, maternal marital status, maternal prenatal alcohol use, and urine specific gravity	MEHP <LOD–613 (min–max) MEHHP 1.1–1,750 MEOHP 0.7–1,320 MECPP 3.0–1,840	BSID-II at 3 years: No significant association with Σ DEHP metabolites in maternal urine in combined or sex-stratified linear regression analyses. No significant associations with Σ DEHP metabolites in maternal urine in combined or sex-stratified logistic regression analyses dichotomizing scores ≤ 85 and > 85 (score ≤ 85 associated with risk of developmental delay on both MDI and PDI).

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Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference and study type	Population	Method of analysis	Metabolite concentration in urine (IQR in ng/mL unless otherwise specified)	Results ^a
Factor-Litvak et al. 2014, Cohort (United States [New York])	328 children (155 boys, 173 girls); members of CCCEH cohort (see Whyatt et al. [2012] above for study population and urine collection). WISC administered to children at 7 years of age.	Linear regression adjusted for specific gravity, maternal IQ, ethnicity, alcohol use during pregnancy, education, marital status, total HOME score, and sex of child	MEHP 1.9–12.4 MEHHP 10.6–47.2	WISC at 7 years: No significant association between full scale or subscale scores and MEHHP or MEHP in maternal urine.
Huang et al. 2015, Cohort (Taiwan)	110 children (58 boys, 52 girls); members of birth cohort (TMICS); mothers 25–34 years of age recruited during 3 rd trimester at medical center between December 1, 2000 and November 30, 2001. Single maternal urine sample collected during 3 rd trimester; children's urine samples collected at ages 2, 5, 8, and 11 years. BSID-II administered at age 2; WPPSI-R at age 5; WISC-III at age 8, and WISC-IV at age 11.	Mixed-model repeat measures analysis adjusted for gender, HOME score, birth weight, maternal education, lactation, and children's age MDI portion of BSID-II used as estimate of IQ in infants	Σ DEHP 58.69 (48.32, 71.30) μ g/g Cr; GM (95% CI) MEHP 19.79 (16.38, 23.92) μ g/g Cr; MEHHP 8.49 (5.97, 12.09) μ g/g Cr MEOHP 12.97(9.23, 18.21) μ g/g Cr	IQ between 2 and 11 years: No significant association with MEHP, MEHHP, or MEOHP in maternal urine. \downarrow IQ with \uparrow MEOHP and Σ DEHP metabolites in child's urine; however, samples were taken at the same time as tests administered.

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Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference and study type	Population	Method of analysis	Metabolite concentration in urine (IQR in ng/mL unless otherwise specified)	Results ^a
Behavioral development				
Whyatt et al. 2012, Cohort (United States [New York])	319 children (151 boys, 168 girls); members of birth cohort (CCCEH); black or Dominican mothers recruited prior to 20 th week of pregnancy (no further details). Single maternal urine sample collected during 3 rd trimester (mean 33 weeks of gestation). Mothers completed CBCL when children were between 33 and 48 months of age (mean 36.6 months).	Linear regression adjusted for child age in months at the time of test administration, child sex, race/ethnicity, maternal IQ; maternal satisfaction with living conditions; maternal perceived hardship; maternal demoralization ^b ; maternal prenatal PAH exposure; maternal prenatal urinary BPA concentrations, and SG	MEHP <LOD–613 (min–max) MEHHP 1.1–1,750 MEOHP 0.7–1,320 MECPP 3.0–1,840	CBCL at 3 years: No significant association with Σ DEHP metabolites in maternal urine in combined or sex-stratified linear regression analyses. No significant association with Σ DEHP metabolites in maternal urine in combined or sex-stratified logistic regression analyses categorizing scores as normal, borderline, or clinical.

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Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference and study type	Population	Method of analysis	Metabolite concentration in urine (IQR in ng/mL unless otherwise specified)	Results ^a										
Gascon et al. 2015b, Cohort (Mexico)	367 children (187 boys, 178 girls); members of birth cohort (INMA or Environment and Childhood); mothers recruited at 1 st trimester prenatal visit at hospital or health center between 2004 and 2006. Maternal urine samples collected at 12 and 32 weeks of gestation and results averaged. CPSCS and ADHD criteria form filled out by teachers when children were age 4. Parents filled out SDQ and short form of CSRS; includes ADHD index) when children were age 7.	Linear regression (CPSCS scores) or negative binomial generalized linear models (ADHD, SDQ, and CSRS) adjusted for sex, maternal age and education, maternal smoking during pregnancy, birth season, breastfeeding, maternal country of origin, number of siblings, and child's age	<table border="1"> <tr> <td>ΣDEHP</td> <td>68–146 µg/g Cr</td> </tr> <tr> <td>MEHP</td> <td>7–17 µg/g Cr</td> </tr> <tr> <td>MEHHP</td> <td>18–41 µg/g Cr</td> </tr> <tr> <td>MEOHP</td> <td>14–30 µg/g Cr</td> </tr> <tr> <td>MECPP</td> <td>27–59 µg/g Cr</td> </tr> </table>	ΣDEHP	68–146 µg/g Cr	MEHP	7–17 µg/g Cr	MEHHP	18–41 µg/g Cr	MEOHP	14–30 µg/g Cr	MECPP	27–59 µg/g Cr	<p>↑ (improved) social competence score at 4 years with ↑ ΣDEHP metabolites in maternal urine β 2.00* 95% CIs not reported</p> <p>↓ (improved) risk of inattention symptoms at 4 and 7 years with ↑ ΣDEHP metabolites in maternal urine IRR [95% CI] = 0.84 [0.72, 0.98]* at 4 years and 0.83 [0.71, 0.95]* at 7 years</p> <p>↓ (improved) risk of ADHD symptoms at 7 years with ↑ ΣDEHP metabolites in maternal urine IRR [95% CI] 0.88 [0.77, 1.00]*</p>
ΣDEHP	68–146 µg/g Cr													
MEHP	7–17 µg/g Cr													
MEHHP	18–41 µg/g Cr													
MEOHP	14–30 µg/g Cr													
MECPP	27–59 µg/g Cr													
Lien et al. 2014, Cohort (Taiwan)	122 children (sex distribution not reported); members of birth cohort (TMICS); mothers 25–34 years of age recruited during 3 rd trimester at medical center between December 1, 2000 and November 30, 2001. Single maternal urine sample collected during 3 rd trimester. Child's urine collected at 8 years of age. Mothers completed CBCL when children were 8 years of age.	Linear and logistic regression adjusted for child's IQ, sex, and family income (logistic regression results shown to right)	<table border="1"> <tr> <td>MEHP</td> <td>16.93 (14.32, 20.02) µg/g Cr (GM [95% CI])</td> </tr> <tr> <td>MEHHP</td> <td>7.91 (5.69, 11.02) µg/g Cr</td> </tr> <tr> <td>MEOHP</td> <td>13.59 (10.27, 18.00) µg/g Cr</td> </tr> </table>	MEHP	16.93 (14.32, 20.02) µg/g Cr (GM [95% CI])	MEHHP	7.91 (5.69, 11.02) µg/g Cr	MEOHP	13.59 (10.27, 18.00) µg/g Cr	<p>CBCL at 8 years: ↑ OR for scores in clinical range (versus normal range) for delinquent behavior with ↑ log-unit ↑ Cr-adjusted MEOHP in maternal urine OR 22.91*</p> <p>↑ OR for scores in clinical range (versus normal range) for aggressive behavior with log-unit ↑ Cr-adjusted MEHP, MEHHP, and MEOHP in maternal urine ORs 9.77*, 4.99*, and 6.88*, respectively</p> <p>↑ OR for scores in clinical range versus normal range) for externalizing problems</p>				
MEHP	16.93 (14.32, 20.02) µg/g Cr (GM [95% CI])													
MEHHP	7.91 (5.69, 11.02) µg/g Cr													
MEOHP	13.59 (10.27, 18.00) µg/g Cr													

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Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference and study type	Population	Method of analysis	Metabolite concentration in urine (IQR in ng/mL unless otherwise specified)	Results ^a
				with log-unit ↑ Cr-adjusted MEHP, MEHHP, and MEOHP in maternal urine ORs 31.01*, 7.41*, and 28.49*, respectively. Significant ORs also seen for borderline or borderline/clinical scores for all three behavior categories and DEHP metabolites. 95% CIs not reported.
Kobrosly et al. 2014, Cohort (United States [California, Minnesota, Missouri, Iowa])	153 children (77 boys, 76 girls) born between 2000 and 2005; members of birth cohort (Study for Future Families); mothers recruited between 1999 and 2005 (no further details). Single maternal urine sample collected between 10 and 39 weeks of gestation (mean 26.6 weeks). Mothers completed CBCL when children were 72–126 months of age (mean 102 months or 8.5 years).	Linear regression adjusted for child sex, child age, mother's education, urinary creatinine, and family stress score	MEHP 1.1, 9.9 MEHHP 6.1, 24.2 MEOHP 5.1, 22.0	↓ (improved) score for anxious/depressed among female children with ↑ ln-transformed ∑DEHP metabolites in maternal urine β -0.21* 95% CI not reported No significant effect on other behavioral scores, or among male children or male and female combined.

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Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference and study type	Population	Method of analysis	Metabolite concentration in urine (IQR in ng/mL unless otherwise specified)	Results ^a
Gender-related play				
Percy et al. 2016, Cohort (United States [Ohio])	227 children (101 boys, 126 girls) born between 2003 and 2006; part of HOME cohort; mothers (16±3 weeks of gestation) recruited at Cincinnati Children's Hospital. Maternal urine samples were collected at 16 and 26 weeks of gestation (on average). Mothers completed GIQ and children completed PPPSI at the child's 8-year clinic visit.	Linear regression analysis for continuous scores, and logistic regression of scores dichotomized by sex at the lower 25 th percentile. Analyses adjusted for race, mother's education, and relational frustration score	ΣDEHP 16 weeks: 87.9 (73.4, 105.3) nmol/L 26 weeks: 65.9 (55.2, 78.5) nmol/L (GM [95% CI]) <hr/> MEHP 16 weeks: 4.9 (4.1, 6) 26 weeks: 4.3 (3.6, 5) <hr/> MEHHP 16 weeks: 27 (22.3, 32.7) 26 weeks: 19.4 (16.1, 23.4) <hr/> MEOHP 16 weeks: 20.1 (16.7, 24.2) 26 weeks: 15.9 (13.2, 19.2) <hr/> MECPP 16 weeks: 39.3 (33, 46.9) 26 weeks: 29.1 (24.5, 34.6)	No association between GIQ or PPPSI scores and mean maternal urinary metabolites in either boys or girls. Odds of having atypical gender-related play were not associated with maternal urinary metabolites in either boys or girls.

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Table 2-15. Summary of Epidemiological Studies of Prenatal DEHP Exposure and Selected Neurodevelopmental Outcomes

Reference and study type	Population	Method of analysis	Metabolite concentration in urine (IQR in ng/mL unless otherwise specified)		Results ^a
Swan et al. 2010 Cohort (United States [California, Minnesota, Missouri, Iowa])	145 children (74 boys, 71 girls) born between 2000 and 2003; members of birth cohort (Study for Future Families); mothers recruited from prenatal clinics between 1999 and 2005. Single maternal urine sample collected midpregnancy. Mothers completed PSAI when children were approximately 5 years old.	Linear regression analysis adjusted for child's age, mother's age, mother's education, parents' attitude towards boy's play, and interaction of mother's education and attribute toward boy's play	Change in PSAI scores for masculine play in boys per log-unit increase in maternal urinary metabolite		
			ΣDEHP	11.7, 40.3	β (95% CI) -3.18 (6.26, 0.10)*
			MEHP	1.4, 6.2	β (95% CI) -0.95 (-3.85, 1.95)
			MEHHP	5.2, 17.3	β (95% CI) -3.29 (-6.14, -0.43)*
			MEOHP	4.7, 17.9	β (95% CI) -2.94 (-5.78, -0.10)*
			No associations were observed between composite or feminine play scores in boys and maternal urinary metabolite levels. No associations were observed between composite, masculine play, or feminine play scores in girls and maternal urinary metabolite levels.		

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

^bDemoralization is defined as "a psychological state characterized by helplessness, hopelessness, a sense of failure and the inability to cope" or a "giving up-given up" complex (Tecuta et al. 2015); maternal demoralization was assessed via questionnaire.

ΣDEHP = sum DEHP metabolites; ADHD = attention-deficit/hyperactivity disorder; BPA = bisphenol A; BSID = Bayley Scales of Infant Development; CBLC = child behavior checklist; CCCEH = Columbia Center for Children's Environmental Health; CI = confidence interval; CPSCS = California Preschool Social Competence Scale; Cr = creatinine; CSRS = Connors' Parent Rating Scales; DEHP = di(2-ethylhexyl)phthalate; ETS = environmental tobacco smoke; GIQ = Gender Identity Questionnaire; GM = geometric mean; HOME = Health Outcomes and Measures of the Environment; INMA = Infancia y Medio Ambiente; IQ = intelligence quotient; IQR = interquartile range; IRR = incidence rate ratio; LOD = limit of detection; max = maximum; 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; min = minimum; MSCA = McCarthy Scales of Children's Abilities; NICU = neonatal intensive care unit; NNNS = NICU Network Neurobehavioral Scale; OR = odds ratio; PAH = polycyclic aromatic hydrocarbon; PDI = Psychomotor Development Index; PPPSI = Playmate and Play Style Preferences Structured Interview; PSAI = pres-School Activities Inventory; SDQ = Strengths and Difficulties Questionnaire; SG = specific gravity; SG-adj = specific gravity adjusted; TMICS = Taiwan Maternal and Infant Cohort Study; WISC = Wechsler Intelligence Scale for Children; WWPSI-R = Wechsler Preschool and Primary Scale of Intelligence-Revised

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cohorts in which prenatal exposure was assessed using maternal urine samples during pregnancy. Both studies were small (137 children in New York and 175 children in Ohio), limiting their power to detect an effect on autism-related behaviors.

The Social Responsiveness Scale (SRS), a validated scale for autistic behaviors, in which a higher score reflects social impairment related to the autism spectrum, is used to assess children's behaviors. In a New York study (Miodovnik et al. 2011), maternal urinary levels of DEHP metabolites measured between 25 and 40 weeks of pregnancy were not associated with scores on the SRS at ages 7–9 years (n=137 children). All regression coefficients adjusted for covariates showed weak positive associations (0.46–0.83) for the total score as well as all subscale scores. In an Ohio study (Braun et al. 2014), when SRS total T-scores were obtained in children 4–5 years old, the regression coefficients between SRS scores and maternal urinary metabolite levels were positive for MEHP and MECPP, whereas MEHHP was not (note that this analysis only used the sensitive covariates in adjustment). Other risk factors (e.g., birth weight; gestational diabetes, depression; Apgar score, birth order; Jeddi et al. 2016) were not considered in either study.

Two cohort studies evaluated potential associations between gender-related play in children and maternal urinary DEHP metabolite levels (Table 2-15). Swan et al. (2010) evaluated whether prenatal DEHP exposure altered the nature of children's play behaviors. In a group of 145 children (74 boys and 71 girls, on average 5 years of age) who were members of a multicenter U.S. birth cohort, prenatal maternal urinary metabolite levels were associated with reduced scores on the Pre-School Activities Inventory (PSAI), indicative of decreased masculine play activities, among boys. In contrast, the U.S. Health Outcomes and Measures of the Environment (HOME) birth cohort 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 in 227 children (101 boys and 126 girls, 8 years old) (Percy et al. 2016). Results from these studies are difficult to compare, primarily due to use of different metrics and different ages at analysis.

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.

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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. No changes in spontaneous locomotion were observed in offspring of CD-1 mouse dams exposed to doses up to 95 mg/kg/day from GD 0 to 17 (Price et al. 1988b).

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 prenatally 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).

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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).

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. (2014b) 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. 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 rats following postnatal exposure to DEHP, including decreased axonal innervation, decreased cell density, decreased dendritic spine density, and reduced neurogenesis (Smith and Holahan 2014; Smith et al. 2011).

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).

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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 PPAR γ 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).

Observed DEHP-moderated alterations in oxidative stress and inflammatory pathways (Ferguson et al. 2012, 2015, 2017; Wu et al. 2017) could potentially contribute to neurodevelopmental toxicity of DEHP; however, the potential role(s) of these pathways has not been specifically evaluated with regard to neurodevelopment.

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, and AGD in infants and children.

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 106 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. (2016b) 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.

Eleven epidemiological studies have investigated the association between reduced AGD in male infants and prenatal DEHP exposure in seven different birth cohorts. Table 2-16 displays the findings of these studies in which prenatal maternal urine samples were used as a biomarker of fetal exposure to DEHP and AGD was measured in infants at various ages between birth and 2 years of age. Associations between

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Table 2-16. Summary of Epidemiological Data on Reproductive Tract Development in Male and Female Infants

Reference, study type, and location	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β) (95% CI), unless otherwise indicated ^a	
Male AGD					
Wenzel et al. 2018, Cohort (United States [South Carolina])	171 male newborns from birth cohort of 193 white and 187 African-American pregnant women (mean maternal age 27.4 years), recruited from the Medical University of South Carolina between 2011 and 2014. Maternal urine sample collected in first trimester for all women (n=380) and again in second trimester for a subset of women (n=219); AGD measured within 48 hours of birth.	Linear regression adjusted for body weight percentile, maternal smoking, race, and education.; a second analysis stratified by race was conducted	Change in anopenile distance (mm) per natural log-increase in SG-adjusted maternal urinary metabolite		
			Σ DEHP	36.3–92.8 nmol/L	-0.93 (-1.96, 0.09)
			MEHP	1.7–5.3	-1.57 (-2.93, -0.20)*
			MEHHP	4.5–12.2	-0.84 (-2.04, 0.35)
			MEOHP	3.8–9.0	-0.99 (-2.29, 0.30)
			Change in anoscrotal distance (mm) per natural log-increase in SG-adjusted maternal urinary metabolite		
			Σ DEHP	36.3–92.8 nmol/L	0.01 (-0.89, 0.91)
			MEHP	1.7–5.3	-0.10 (-1.31, 1.11)
			MEHHP	4.5–12.2	0.04 (-1.00, 1.09)
			MEOHP	3.8–9.0	-0.48 (-1.79, 0.82)
In the analyses stratified by race, larger (but still not significant, except for MEHP) coefficients were observed for African-American infants, but the interaction term for race x phthalates was not statistically significant.					
Adibi et al. 2015; Barrett et al. 2016; Martino-Andrade et al. 2016; Swan et al. 2015, Cohort (United States [Minnesota,	366 male newborns from birth cohort of 738 pregnant women in four states (TIDES; mean maternal age 31.1 years), recruited from prenatal clinics from 2010 to 2012. Maternal urine sample collected in first trimester; AGD measured shortly after birth.	Linear regression adjusted for infant age at exam, gestational age at birth, study center, weight-for-length z-score, specific gravity, time of day of urine collection, maternal age, maternal race, and maternal first	Change in anopenile distance (mm) per natural log-increase in maternal urinary metabolite		
			Σ DEHP	71.7 (65.6–78.3) ^b nmol/L	-1.35 (-2.65, -0.05)*
			MEHP	1.93 (1.76–2.11) ^b	-1.21 (-2.41, 0.00)*
			MEHHP	6.04 (5.49–6.64) ^b	-1.29 (-2.28, -0.29)*
			MEOHP	4.22 (3.84–4.63) ^b	-1.6 (-2.81, -0.38)*
			MECPP	8.12 (7.42–8.89) ^b	-0.94 (-2.26, 0.37)
			Change in anoscrotal distance (mm) per natural log-increase in maternal urinary metabolite		
			Σ DEHP	71.7 (65.6–78.3) ^b nmol/L	-1.26 (-2.38, -0.15)*

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Table 2-16. Summary of Epidemiological Data on Reproductive Tract Development in Male and Female Infants

Reference, study type, and location	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β) (95% CI), unless otherwise indicated ^a
California, New York, Washington]	and prenatal stress. Martino-Andrade et al. (2016) evaluated a subset of this cohort that had maternal urine samples for first, second, and third trimesters. Adibi et al. (2015) evaluated the same endpoint for a subset of the cohort (mothers with hCG measurements).	trimester life event stress	MEHP 1.93 (1.76–2.11) ^b	-1.14 (-2.18, -0.11)*
			MEHHP 6.04 (5.49–6.64) ^b	-1.47 (-2.62, -0.31)*
			MEOHP 4.22 (3.84–4.63) ^b	-1.44 (-2.48, -0.4)*
			MECPP 8.12 (7.42–8.89) ^b	-0.97 (-2.09, 0.16)
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.				
Jensen et al. 2016, Cohort (Denmark)	273 male infants (3 months old) from Odense Child Cohort; pregnant women recruited between 8 and 16 weeks of gestation; maternal urine sample collected at 26–30 weeks of gestation; AGD measured in offspring at 3 months of age.	Multivariate linear regression adjusted for postconceptional age (sum of gestational age at birth and age at AGD measurement), and weight-for-age z-score	Change in anopenile distance (mm) between highest and lowest quartiles in osmolality-adjusted maternal urinary metabolite concentration	
			Σ DEHP (MEHP, MEHHP, MEOHP, MECPP) 11.4–36.1 (molar sum expressed as excreted DEHP)	-0.45 (-2.56, 1.66)
			Change in anoscrotal distance (mm) between highest and lowest quartiles in osmolality-adjusted maternal urinary metabolite concentration	
			Σ DEHP 11.4–36.1 (molar sum expressed as excreted DEHP)	-1.16 (-3.08, 0.77)
			MEHP 0.4–2.3 (osmolality-adjusted)	NR
			MEHHP 2.4–9.1	NR
			MEOHP 2.2–7.1	NR
MECPP 2.7–8.7	NR			
Sum DEHP metabolite concentration in 1 st quartile: LOD–13.9 ng/mL; 4 th quartile: \geq 34 ng/mL				

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Table 2-16. Summary of Epidemiological Data on Reproductive Tract Development in Male and Female Infants

Reference, study type, and location	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β) (95% CI), unless otherwise indicated ^a	
Bornehag et al. 2015, Cohort (Sweden)	196 male infants from SELMA birth cohort (n=2,000 mother-child pairs recruited during 10 th week of gestation), born between August 2009 and November 2010. Maternal urine measured at recruitment (9–11 weeks of gestation); AGD measured at mean age 20.8 months.	Linear regression, adjusted for infant age, gestational week of urine sampling, weight-for-age percentile, and creatinine	Change in anopenile distance (mm) per log-unit increase in maternal urinary metabolite		
			Σ DEHP	84.56–220.71 nmol/L	-1.39 (-4.49, 1.70)
			MEHP	1.91–5.86	-1.74 (-4.43, 0.95)
			MEHHP	8.69–22.85	-1.5 (-4.5, 1.49)
			MEOHP	5.67–15.60	-1.25 (-4.19, 1.70)
			MECPP	8.00–22.50	-0.64 (-3.69, 2.40)
			Increase in anoscrotal distance (mm) per log-unit increase in maternal urinary metabolite		
			Σ DEHP	84.56–220.71 nmol/L	-1.16 (-4.01, 1.68)
			MEHP	1.91–5.86	-1.28 (-3.74, 1.17)
			MEHHP	8.69–22.85	-1.24 (-3.99, 1.51)
			MEOHP	5.67–15.60	-0.77 (-3.48, 1.94)
			MECPP	8.00–22.50	-0.89 (-3.69, 1.95)
			In analyses using categorized AGD values (by quartile), the odds of infant with AGD <25 th percentile was increased with log-transformed DEHP metabolite levels in maternal urine (ORs 1.47–1.82) but the increases were not statistically significant.		

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Table 2-16. Summary of Epidemiological Data on Reproductive Tract Development in Male and Female Infants

Reference, study type, and location	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β) (95% CI), unless otherwise indicated ^a	
Bustamante-Montes et al. 2013, Cohort (Mexico)	73 male infants from birth cohort (pregnant women >18 years of age, mean age 29.5 years, recruited in last trimester of pregnancy at single hospital). Maternal urine samples collected at recruitment; AGD measured 24–48 hours after birth.	Linear regression adjusted for creatinine and supine length at birth	Association between anoscrotal distance (mm) and maternal urinary metabolite level		
			MEHP	0.4–19.5	-0.0049 (NR)
			Association between distance from anus to posterior base of penis (mm) and maternal urinary metabolite level		
			MEHP	0.4–19.5	-0.0733 (NR)
			Association between distance from anus to posterior base of penis (mm) and maternal urinary metabolite level		
			MEHP	0.4–19.5	-0.0252 (NR)
Suzuki et al. 2012, Cohort (Japan)	111 male infants from a birth cohort of 224 mother-infant pairs who delivered at the Central Hospital of the Defense Force in Tokyo. Maternal urine samples collected at mean 29 weeks of gestation (range 9–40 weeks). AGD measured at birth.	Multiple linear regression adjusted for phthalate metabolite, maternal partner smoking, gestational week, birth order, maternal age, and log-transformed maternal urinary isoflavone concentrations	Change in body-weight corrected distance from anus to anterior genitalia (mm/kg) per log-unit increase in SG-adj maternal urinary metabolite		
			Nonsmoking mothers (n=107)		
			MEHP	2.92–8.03	-0.246 (-0.435, -0.057)*
			MEHHP	6.79–14.4	NR
			MEOHP	6.92–15.2	NR
			Smoking and non-smoking mothers (n=111)		
			MEHP	2.92–8.03	-0.226 (-0.41, -0.042)*
MEHHP	6.79–14.4	NR			
			MEOHP	6.92–15.2	NR

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Table 2-16. Summary of Epidemiological Data on Reproductive Tract Development in Male and Female Infants

Reference, study type, and location	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β) (95% CI), unless otherwise indicated ^a	
Swan 2008, Cohort (United States [Minnesota, Missouri, California])	106 male infants from birth cohort (Study for Future Families multi-center cohort). Maternal urine samples collected at mean gestation week 28.6; average infant age at AGD measurement was 12.8 months.	Mixed model regression adjusted for age and weight percentile	Association between distance from center of anus to cephalad base of penis (mm) and log-transformed maternal metabolite concentration		
			MEHP	Short AGD: 6.2 (median) Intermediate AGD: 2.9 Long AGD: 2.3	-3.503* (NR)
			MEHHP	Short AGD: 19.8 Intermediate AGD: 10.0 Long AGD: 8.2	-4.977* (NR)
			MEOHP	Short AGD: 21.3 Intermediate AGD: 11.7 Long AGD: 7.3	-5.126* (NR)
Swan et al. 2005 reported previous analysis of this cohort (smaller n)			MECPP	NA	NA
Hydrocele, hypospadias, and cryptorchidism					
Sathyanarayana et al. 2016b Cohort (United States [Minnesota, California, New York, Washington])	371 male newborns from birth cohort of pregnant women in four states (TIDES), recruited at 2010–2012. Maternal urine sample collected in first trimester; genital anatomical anomalies evaluated during physical exam at birth. In total, 37/371 male infants had a genital anomaly (5 cryptorchidism, 30 hydrocele, 3 hypospadias, and 4 with multiple anomalies).	Logistic regression adjusted for study center, maternal age, birth weight, and age at exam	Risk of male genital anomaly per log-unit increase in SG-adjusted maternal urinary metabolite concentration ($\mu\text{g/L}$)		
			Σ DEHP	14.86–38.80 nmol/L	OR 2.54 (1.09, 5.92)*
			MEHP	1.28–3.63	OR 2.49 (1.13, 5.50)*
			MEHHP	3.76–11.24	OR 2.52 (1.16, 5.46)*
			MEOHP	2.54–7.25	OR 2.49 (1.11, 5.58)*
			MECPP	6.42–16.21	OR 2.34 (0.96, 5.69)
			Risk of hydrocele per log-unit increase in SG-adjusted maternal urinary metabolite concentration ($\mu\text{g/L}$)		
			Σ DEHP	14.86–38.80 nmol/L	OR 3.01 (1.19, 7.62)*
			MEHP	1.28–3.63	OR 3.13 (1.31, 7.48)*
			MEHHP	3.76–11.24	OR 3.17 (1.35, 7.74)*
MEOHP	2.54–7.25	OR 3.17 (1.30, 7.73)*			

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Table 2-16. Summary of Epidemiological Data on Reproductive Tract Development in Male and Female Infants

Reference, study type, and location	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β) (95% CI), unless otherwise indicated ^a
			MECPP 6.42–16.21	OR 2.52 (0.94, 6.77)
			Risk of hypospadias or cryptorchidism per log-unit increase in SG-adjusted maternal urinary metabolite concentration ($\mu\text{g/L}$)	
			Σ DEHP 14.86–38.80 nmol/L	OR 0.88 (0.09, 8.44)
			MEHP 1.28–3.63	OR 0.74 (0.09, 6.10)
			MEHHP 3.76–11.24	OR 0.77 (0.11, 5.44)
			MEOHP 2.54–7.25	OR 0.60 (0.07, 5.13)
			MECPP 6.42–16.21	OR 1.23 (0.13, 11.46)
			When evaluated by quartile of log-transformed SG-adjusted urinary metabolite concentrations, only the third quartile of MEHHP showed a statistically significant increase in odds for any male reproductive anomaly, OR: 3.89 (1.16, 13.04) (graphically reported data).	
Chevrier et al. 2012, Nested case-control (France)	21 cases of hypospadias, 50 cases of cryptorchidism, and (for each) 3:1 control male infants matched on residence, and gestational age, day, and date of urine collection; members of two birth cohorts (EDEN and PELAGIE) of pregnant women recruited before the 28 th week of pregnancy. Maternal urine samples collected between 6 and 19 weeks of gestation in PELAGIE cohort and between 24 and 30 weeks in the EDEN cohort. Case status determined at birth.	Conditional logistic regression, adjusted for maternal age, parity, educational level, gestational duration, and creatinine	OR for hypospadias comparing highest and lowest tertiles of maternal urinary metabolite concentration	
			Σ DEHP NR (MEHP, MEHHP, MEOHP, MECPP)	0.21 (0.04, 2.1)
Philippat et al. 2012			OR for cryptorchidism comparing highest and lowest tertiles of maternal urinary metabolite concentration	
			Σ DEHP NR	0.6 (0.2, 1.7)
			MEHP 0.8–40.7 (5 th –95 th percentile)	NR
			MEHHP 4.6–147.0	NR
			MEOHP 3.6–112.0	NR
			MECPP 11.6–183.0	NR

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Table 2-16. Summary of Epidemiological Data on Reproductive Tract Development in Male and Female Infants

Reference, study type, and location	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β) (95% CI), unless otherwise indicated ^a	
Swan 2008, Cohort (United States [Minnesota, Missouri, California])	106 male infants from birth cohort (Study for Future Families multi-center cohort). Maternal urine samples collected at mean gestation week 28.6; average infant age at testicular descent determination was 12.8 months.	Logistic regression adjusted for age and weight percentile	Association between probability of normal testicular descent and log-transformed maternal metabolite concentration		
			Σ DEHP	NR	-1.447 (NR)
			MEHP	Short AGD: 6.2 (median) Intermediate AGD: 2.9 Long AGD: 2.3	-1.258* (NR)
			MEHHP	Short AGD: 19.8 Intermediate AGD: 10.0 Long AGD: 8.2	-1.417 (NR)
Swan et al. 2005 reported previous analysis of this cohort (smaller n)			MEOHP	Short AGD: 21.3 Intermediate AGD: 11.7 Long AGD: 7.3	-1.350 (NR)
Female AGD					
Wenzel et al. 2018, Cohort (United States [South Carolina])	128 female newborns from birth cohort of 193 white and 187 African American pregnant women (mean maternal age 27.4 years), recruited from the Medical University of South Carolina between 2011 and 2014. Maternal urine sample collected in first trimester for all women and again in second trimester for 219 women; AGD measured within 48 hours of birth.	Linear regression adjusted for body weight percentile, maternal age, smoking, race, and education; a second analysis stratified by race was conducted	Change in anoclitral distance (mm) per natural log-increase in SG-adjusted maternal urinary metabolite		
			Σ DEHP	36.3–92.8 nmol/L	-0.71 (-1.69, 0.27)
			MEHP	1.7–5.3	-1.17 (-2.60, 0.26)
			MEHHP	4.5–12.2	-0.94 (-2.18, 0.29)
			MEOHP	3.8–9.0	-0.86 (-2.30, 0.58)
			Change in anofourchette distance (mm) per natural log-increase in SG-adjusted maternal urinary metabolite		
			Σ DEHP	36.3–92.8 nmol/L	-0.38 (-0.99, 0.23)
			MEHP	1.7–5.3	-0.69 (-1.58, 0.20)
MEHHP	4.5–12.2	-0.48 (-1.24, 0.29)			
MEOHP	3.8–9.0	-0.49 (-1.39, 0.40)			

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Table 2-16. Summary of Epidemiological Data on Reproductive Tract Development in Male and Female Infants

Reference, study type, and location	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Regression coefficient (β) (95% CI), unless otherwise indicated ^a	
Similar results were observed in the analysis stratified by race; the interaction term for race x phthalates was not statistically significant.					
Adibi et al. 2015; Barrett et al. 2016; Swan et al. 2015, Cohort (United States [Minnesota, California, New York, Washington])	373 female newborns from birth cohort of 738 pregnant women in four states (TIDES; mean maternal age 31.1 years), recruited from prenatal clinics from 2010 to 2012. Maternal urine sample collected in first trimester; AGD measured shortly after birth.	Linear regression adjusted for infant age at exam, gestational age at birth, study center, weight-for-length z-score, specific gravity, time of day of urine collection, maternal age, maternal race, and maternal first trimester life event stress	Change in anoclitral distance (mm) per natural log-increase in urinary metabolite		
			Σ DEHP	71.7 (65.6–78.3) ^b nmol/L	-0.34 (-1.4, 0.72)
			MEHP	1.93 (1.76–2.11) ^b	-0.15 (-1.07, 0.78)
			MEHHP	6.04 (5.49–6.64) ^b	-0.3 (-1.29, 0.68)
			MEOHP	4.22 (3.84–4.63) ^b	0.01 (-1.01, 1.02)
			MECPP	8.12 (7.42–8.89) ^b	-0.44 (-1.41, 0.54)
			Change in anofourchette distance (mm) per natural log-increase in urinary metabolite		
			Σ DEHP	71.7 (65.6–78.3) ^b nmol/L	0.29 (-0.54, 1.13)
			MEHP	1.93 (1.76–2.11) ^b	0.05 (-0.68, 0.78)
			MEHHP	6.04 (5.49–6.64) ^b	0.27 (-0.51, 1.04)
MEOHP	4.22 (3.84–4.63) ^b	0.33 (-0.47, 1.13)			
MECPP	8.12 (7.42–8.89) ^b	0.26 (-0.51, 1.03)			

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

^bGM (95% CI).

Σ DEHP = sum DEHP metabolites; AGD = anogenital distance; CI = confidence interval; DEHP = di(2-ethylhexyl)phthalate; GM = geometric mean; hCG = human chorionic gonadotropin; IQR = interquartile range; 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; NA = not applicable; NR = not reported; OR = odds ratio; SELMA = Swedish Environmental Longitudinal Mother and child Asthma and Allergy; SG-adj = specific gravity adjusted; TIDES = The Infant Development and Environment Study

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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 the remaining three cohorts (Bornehag et al. 2015; Bustamante-Montes et al. 2013; Jensen et al. 2016) also suggested 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.

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. An association (β -0.782; $p=0.005$) between decreased penile width and log-transformed prenatal MEHP (but not other metabolites) was seen in 1-year-old boys ($n=106$) in the United States (Swan 2008). However, no association between penile width and DEHP metabolites was observed in newborns ($n=73$) in Mexico (Bustamante-Montes et al. 2013) or infants ($n=273$) 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 (β -0.2604; $p=0.05$); however, no other studies are available to corroborate this finding.

Two studies (Su et al. 2015; Ferguson et al. 2014c) examined the relationship between timing of puberty in boys and maternal DEHP exposure, with inconsistent results. In 115 boys 8–14 years old, Ferguson et al. (2014c) observed a decrease in the OR (0.12; $p=0.05$) for presence of pubic hair with an interquartile range increase in prenatal MEHHP, but the OR for development of genitalia was not associated with prenatal exposure levels. Testicular volume was not associated with DEHP exposure measures in this study or in the study by Su et al. (2015) of 122 boys that were 8 and 11 years old.

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

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134 boys ages 6–12 years (percent change -29.3; 95% CI -46.8, -6.10 for Σ DEHP). No other data on serum testosterone 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- to 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). Testicular lesions were also observed at maternal doses ≥ 3 mg/kg/day in Long-Evans rat offspring exposed to DEHP during gestation and lactation (Arcadi et al. 1998). 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*

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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 $\geq 1,040$ mg/kg/day, but not ≤ 380 mg/kg/day, in 2-generation studies in Wistar rats (Schilling et al. 1999, 2001). 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.

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).

Alterations in male reproductive organ histology have also been reported in neonatal and weanling 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

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(PNDs 6–10 or 14–18) or post-weaning (PNDs 21–25 or 42–46) at doses $\geq 1,000$ mg/kg/day, but not ≤ 100 mg/kg/day; rats were sacrificed 24 hours after the final dose (Dostal et al. 1988). However, Li et al. (2000) reported altered morphology of germ cells (gonocytes were enlarged and multinucleated) and reduced Sertoli cell proliferation in male Sprague-Dawley rats 24 hours after a single exposure to DEHP on PND 3 at ≥ 100 mg/kg/day, but not 20 mg/kg/day. 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. Noriega et al. (2009) also reported hypospermia and testicular and epididymal degeneration in weanling Sprague-Dawley rats, but only at exposure levels ≥ 300 mg/kg/day, and 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).

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 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), 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 $\geq 1,000$ mg/kg/day resulted in decreased testes weights, but doses ≤ 100 mg/kg/day did not (Dostal et al. 1988). 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 mg/kg/day in another (Dalsenter et al. 2006). 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). Only two 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

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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 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. Decreased organ weights were observed in offspring of rats and mice exposed to DEHP during gestation and lactation, including ventral prostate and LABC muscles in Wistar rats at ≥ 10 mg/kg/day (Christiansen et al. 2010); ventral prostate and seminal vesicles in Wistar rats at 500 mg/kg/day (Dalsenter et al. 2006); glans penis, ventral prostate, seminal vesicles, LABC muscles, Cowper's glands, and epididymides at 300 mg/kg/day (Gray et al. 2009); and seminal vesicles at ≥ 0.05 mg/kg/day (Pocar et al. 2012). 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) or 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).

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). 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). 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). The significance of this non-monotonic response is unclear.

Decreased AGD, suggesting demasculinization, has been reported in male offspring following 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, but not doses up to 100 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, but not doses up to 10 mg/kg/day (Lin et al. 2009). In Sprague-Dawley rats, AGD was significantly decreased at PND 2 following gestational and lactational exposure to ≥ 300 mg/kg/day, but not at doses up to 135 mg/kg/day (Andrade et al. 2006c;

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Gray et al. 2009). Decreased AGD was observed in PND 63 male offspring of Sprague-Dawley rat dams exposed to DEHP from GD 11 to 21 at ≥ 100 mg/kg/day. In 2-generation studies in Wistar rats, both AGD and the anogenital index (AGI; corrected for body weight) were significantly decreased on PND 1 or 2 in both F1 and F2 males at doses ≥ 340 mg/kg/day in one study (Schilling et al. 2001), but not until doses of 1,040 mg/kg/day in another (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). However, a gestation/lactation exposure study in Wistar rats reported decreased AGD in male pups at PND 1 at doses ≥ 10 mg/kg/day (Christiansen et al. 2010). Decreased AGD 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). However, no exposure-related changes in AGD were observed in CD-1 fetuses on GD 18 following maternal exposure to doses up to 500 mg/kg/day from GD 9 to 18 (Do et al. 2012). No changes in PND 42 AGD were observed in CD-1 mice following maternal exposure to low doses of DEHP (≤ 5 mg/kg/day) during gestation and lactation (Pocar et al. 2012).

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. BMD₅ 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). 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

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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 offspring following maternal exposure to doses up to 500 mg/kg/day during gestation and lactation (Dalsenter et al. 2006; Gray et al. 2009). A subset of the offspring also received direct DEHP exposure from PND 18 to 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). 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 (Price et al. 1988b). 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.

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.

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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). 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). 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 or 100 mg/kg/day, respectively (Dalsenter et al. 2006; Lin et al. 2009). No exposure-related changes in serum testosterone or estradiol were observed in adult male offspring following maternal exposure to doses up to 300 mg/kg/day during gestation and lactation in Sprague-Dawley rats (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).

Observed alterations in male reproductive hormones following exposure to DEHP in weanlings 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). The significance of this non-monotonic response is unclear without further study of the pituitary-testes axis. 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, but no changes in serum hormone levels were observed (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

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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).

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 BMD₅ value of 15 mg/kg/day was calculated. In addition, an alternate BMD₄₀ 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).

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Inhibition of steroidogenic enzyme activities was also observed in rats exposed for 28 days, including reduced 17 β -hydroxysteroid dehydrogenase (17 β -HSD) at ≥ 10 mg/kg/day, reduced P450_{scc} and 3 β -HSD at ≥ 100 mg/kg/day, and reduced P45017 α 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.

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 Jugou 2014). 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-Müllerian hormone) (Albert and Jugou 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 Beaman 1984; Gray and Gangolli 1986; Sjöberg et al. 1986), decreased germ cell viability (Gray and Beaman 1984), elongation of Sertoli cells without evidence of decreased viability (Gray and Beaman 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

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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 two pregnancy cohorts (Adibi et al. 2015; 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 either cohort (Table 2-16).

The timing of puberty has been examined in two studies using urinary biomarkers of DEHP exposure measured prior to outcome evaluation; results were mixed (Watkins et al. 2014; Wolff et al. 2014). In a Mexican birth cohort, evaluation of pubertal development in 129 girls aged 8–13 years showed an association between early development of puberty and third-trimester maternal levels of MEHP (Watkins et al. 2014). The OR for pubic hair development associated with an IQR increase in ln-MEHP in maternal urine was 5.30 (95% CI 1.13, 24.95, after adjustment for age, BMI Z-score, and specific gravity). In contrast, a study that examined childhood urinary metabolite levels and subsequent pubertal onset over 7 years of follow-up observed a relationship between *delayed* puberty and DEHP exposure (Wolff et al. 2014). In this cohort of 1,239 girls in New York, Ohio, and California, urinary levels of DEHP metabolites at ages 6–8 years were associated with reduced hazard ratios (HRs) for age at first pubic hair development (HR 0.79, 95% CI 0.64, 0.98 comparing highest quintile of total DEHP metabolites, after adjustment for covariates), especially among normal weight girls (HR 0.70, 95% CI 0.53, 0.93). Associations were not observed between DEHP metabolites and menarche (Watkins et al. 2014) or breast development (Watkins et al. 2014; Wolff et al. 2014). The use of a single urine sample to estimate exposure is a significant limitation in these studies.

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

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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, Grande et al. (2006) did not observe any changes in female AGD at PND 22 following gestational and lactational exposure to doses up to 405 mg/kg/day. 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). 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, 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 adult female offspring similarly exposed, 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). 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,

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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 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. 2015; Zhang et al. 2013b, 2015). In addition to interaction with ERs (Mu et al. 2015; 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. 2015). 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 may cause heritable epigenetic alterations in germ cells, which may contribute to altered ovarian development (Li et al. 2014; Zhang et al. 2013b, 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 maternally imprinted genes for insulin like growth factor 2 receptor (*Igf2r*) and paternally expressed 3 (*Peg3*) (Li et al. 2014).

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Animal Studies—Other Noncancer (Glucose/Insulin Homeostasis). Altered glucose homeostasis has been observed in Wistar rats following developmental exposure to DEHP during gestation, gestation plus lactation, or lactation only at doses ≥ 1 mg/kg/day (Lin et al. 2011; Mangala Priya et al. 2014; Rajesh and Balsubramanian 2014a). Insulin sensitivity, but not altered glucose tolerance, was observed in mice exposed to DEHP during gestation plus lactation at a maternal dose of 500 mg/kg/day (Hunt et al. 2017). Findings from these studies are discussed below. A NOAEL for altered glucose homeostasis following developmental DEHP exposure has not been identified. This endpoint has not been assessed in other strains or species.

Adult offspring of Wistar rats exposed to DEHP at doses ≥ 1 mg/kg/day (lowest dose tested) during gestation (GDs 9–21) 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 Balsubramanian 2014a). Additional significant findings observed in adult offspring included decreased glycogen content and decreased insulin binding, glucose uptake, and glucose oxidation in skeletal muscle. Several genes or gene products involved in insulin signaling were dysregulated in adult offspring, including decreased glucose transporter 4 (*GLU4*) gene expression, increased *GLU4* phosphorylation (posttranslational modification that decreases activity), and epigenetic silencing of *GLU4* (Rajesh and Balsubramanian 2014a).

Altered glucose homeostasis, along with pancreatic dysfunction, was also observed in weanling and adult offspring of Wistar rats exposed to DEHP at 1.25 or 6.25 mg/kg/day during gestation and lactation (GD 0–PND 21) (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

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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. Alterations in mRNA expression of genes essential for pancreatic β -cell function were observed, including downregulation of *Pdx-1* and upregulation of *Atf4*, *Atf6*, *Bip*, and *Ucp2*. 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.

Lactational exposure to DEHP from PND 1 to 21 via maternal doses ≥ 1 mg/kg/day also resulted in altered glucose homeostasis in adult female Wistar rat offspring (male offspring were not assessed) (Mangala Priya et al. 2014). Fasting blood glucose was significantly elevated by ~ 15 – 20% (data presented graphically) in PND 59 female offspring. Glucose uptake and oxidation were also significantly decreased in cardiac tissue, and protein expression analysis showed altered expression of insulin signaling molecules that could account for decreased glucose uptake into cardiac tissue.

Insulin sensitivity was observed in PNW 16 wild-type 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.

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; Kobayashi et al. 2006; Martinez-Arguelles et al. 2011, 2013; Piepenbrink et al. 2005; Wei et al. 2012), but data are

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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 has also been reported following developmental exposure. Fetotoxic and teratogenic effects have been observed at higher exposure levels following gestational exposure in animals.

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Epidemiology Studies. Several cross-sectional studies that used urinary metabolite levels to assess DEHP exposure (Table 2-17) have reported associations with increased fasting blood glucose (Huang et al. 2014a) or insulin resistance (as assessed by homeostatic model assessment-insulin resistance [HOMA-IR]; Lin et al. 2016; Attina and Trasande 2015; James-Todd et al. 2012; Trasande et al. 2013a). 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. 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. No association between fasting blood glucose and DEHP metabolite levels in urine was observed in a study of 250 Mexican children aged 8–14 years (Watkins et al. 2016).

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

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

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Indices of glucose homeostasis					
Lin et al. 2016, Cross-sectional (Taiwan)	243 male and 550 female students (mean age 21.28 years), including 303 with and 486 without elevated blood pressure in childhood, from the YOTA study (recruited 1992–2000 from schools). Timing of blood and urine samples was not clearly reported.	Linear regression adjusted for age, gender, and smoking status	Association between log-HOMA-IR and log-transformed Cr-adjusted urinary metabolite concentration		
			MEHP	6.1 (5.1–7.32) µg/g Cr (GM [95% CI])	β 0.071* (NR)
			MEHHP	27.90 (26.05–29.96) µg/g Cr	β -0.015 (NR)
			MEOHP	17.48 (16.44–18.54) µg/g Cr	β -0.007 (NR)
Watkins et al. 2016, Cross-sectional (Mexico)	250 children (age 8–14 years) from birth cohort (ELEMENT); mothers recruited 1997–2004 from maternity hospitals. Children provided contemporaneous blood and urine samples during followup.	Linear regression adjusted for age, BMI z-score, and urinary specific gravity	Percent difference in fasting serum glucose (mg/dL) per IQR increase in child's ln-transformed urinary metabolite concentration		
			Prepubertal boys (n=56)		
			ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	3.09–10.3 µmol/L	Difference 3.0 (-1.4, 7.5)
			Prepubertal girls (n=86)		
			ΣDEHP (see above)	3.09–10.3 µmol/L	Difference -0.04 (-4.8, 5.0)
			Pubertal boys (n=58)		
			ΣDEHP (see above)	3.09–10.3 µmol/L	Difference 1.0 (-1.4, 3.5)

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

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
			Pubertal girls (n=45)	
			ΣDEHP 3.09–10.3 μmol/L (see above)	Difference 1.9 (-1.8, 5.6)
Attina and Trasande 2015, Cross-sectional (United States)	356 adolescents (12–19 years); 191 males and 165 females; participants in NHANES 2009–2012. Insulin resistance defined as HOMA-IR >4.39. Urine and blood samples collected contemporaneously. n=350 in adjusted analyses.	Linear regression, Multivariate Logistic regression (considering these covariates: urinary creatinine, age, caloric intake, sex, poverty-income ratio, serum cotinine, BMI, race/ethnicity categories, and physical activity)	OR for insulin resistance (HOMA-IR >4.39) comparing highest and lowest tertiles of log-transformed urinary metabolite concentration ΣDEHP 0.07–0.32 μM	OR 3.85 (1.6, 9.24)*
			Difference in HOMA-IR between highest and lowest tertiles of log-transformed urinary metabolite concentration ΣDEHP 0.07–0.32 μM	Difference 0.16 (-0.005, 0.33)
			OR for insulin resistance (HOMA-IR >4.39) per log-unit increase in urinary metabolite concentration	
			MEHP NR	OR 1.52 (1.15, 2.00)*
			MEHHP NR	OR 1.64 (1.25, 2.15)*
			MEOHP NR	OR 1.68 (1.25, 2.26)*
			MECPP NR	OR 1.60 (1.19, 2.14)*
Dirinck et al. 2015, Cross-sectional (Belgium)	123 adult obese subjects (38 men, 85 women) without a history of type 2 diabetes recruited from weight management clinic of Antwerp University Hospital between November 2009 and February 2012; mean	Linear regression adjusted for gender, age, physical activity level, current smoking behavior, current medication use, and BMI	Association with log-transformed creatinine-adjusted urinary metabolite concentration MEHP 0.49–181.9 μg/g Cr (min–max) MEHHP 2.6–135.8 μg/g Cr	NR (not significant) Log-Belfiore index β -0.133 (-0.265, 0.00)* Square root-AUC Insulin β 25.199 (-0.736, 51.134) Log-Matsuda index NR (not significant)

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

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
	age±SD = 41±12.5. Urine samples collected 1 day before blood samples.		MEOHP 0.82–42.3 µg/g Cr	Log-Belfiore index β -0.143 (-0.275, -0.011)* Square root-AUC Insulin β 26.617 (0.692, 52.541)* Log-Matsuda index β -0.199 (-0.383, -0.014)*
	Belfiore index is an estimate of insulin resistance; higher values indicate increased resistance. Matsuda index is an estimate of insulin sensitivity; lower values indicate increased insulin resistance.		MECPP 0.1–268.8 µg/g Cr	NR (not significant)
			No significant association between any urinary DEHP metabolites and HbA1c levels, AUC glucose, HOMA-IR, or insulinogenic index.	
Huang et al. 2014a, Cross-sectional (United States)	3,083 non-diabetic, non-pregnant subjects; 1,620 men, 1,463 women; ages 12–<80 years; participants in NHANES 2001–2008	Median regression adjusted age, sex, race, urinary creatinine, fasting time, total caloric intake, triglyceride, education, smoking status, and poverty	Median difference between highest and lowest quartiles of urinary metabolite concentration ΣDEHP (MEHP, MEHHP, MEOHP) Men: 5.3–19.7 µmol/100 g Cr; Women: 6.5–23.1 µmol/100 g Cr	Median fasting blood glucose Difference = 2.45 (1.29, 3.60)* Median HOMA-IR Difference = 0.68 (0.47, 0.88)*
			No significant change in median fasting blood glucose for middle quartiles; p for trend = 0.0016. Significant changes in median HOMA-IR for 2 nd and 3 rd quartiles; p for trend <0.0001.	

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

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Kim and Hong 2014; Kim et al. 2013, Panel study (Korea)	560 subjects (146 men, 414 women; 60–87 years); participants in Korean Elderly Environmental Panel study; recruited from a community elderly welfare center, where they underwent medical examinations up to 5 times, with blood and urine samples collected on the same day at least 3 times.	Linear regression adjusted for age, BMI, education, exercise, cotinine levels, PM ₁₀ , O ₃ , NO ₂ , outdoor temperature, dew point, total caloric and fat intake, history of diabetes mellitus (in women only analysis), and sex (in analysis of men and women)	Change per log-change in Cr-adjusted urinary metabolite concentration		
			Women only		
			ΣDEHP	NR	Fasting serum glucose β 0.11 (-0.003, 0.22) HOMA-IR β 0.3 (0.04, 0.56)*
			MEHHP	1.71–317.26 (min–max)	NR
			MEOHP	0.212–231.44	NR
			Men and women		
		ΣDEHP	NR	Fasting serum glucose β 0.11 (0.01, 0.22)* HOMA-IR β 0.26 (0.01, 0.51)*	
	Kim and Hong (2014) analyzed data on women only; Kim et al. (2013) analyzed data on men and women.		MEHHP	12.51–39.93	NR
			MEOHP	9.54–33.14	NR
James-Todd et al. 2012, Cross-sectional (United States)	215 female cases of self-reported diabetes (type not specified), 2,135 women without diabetes (ages 20–79 years); participants in NHANES 2001–2008. Urine and blood samples collected at the same time.	Linear regression adjusted for urine creatinine, age, race/ethnicity, education, poverty status, fasting time, total caloric intake, total fat intake, smoking status, physical activity, BMI, and waist circumference	Difference in between highest and lowest quartiles of urinary metabolite concentration.		
			ΣDEHP	1,110 (1,030, 1,200) (units not reported; GM [95% CI])	Median fasting blood glucose (mg/dL) Difference = 0.01 (-2.34, 2.36)
			(MEHP, MEHHP, MEOHP)		Median A1c (%) Difference = -0.02 (-0.07, 0.03)
					Median ln-HOMA-IR Difference = 0.13 (0.01, 0.25)*

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

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Trasande et al. 2013a, Cross-sectional (United States)	766 adolescents (424 males and 342 females; 12–19 years of age); participants in NHANES 2003–2008. Urine and blood samples collected at the same time.	Logistic regression adjusted for urinary creatinine, age, BMI, gender, poverty-income ratio, parental education, serum cotinine, and race/ethnicity	OR for HOMA-IR >2 SD above mean per log-unit increase in urinary metabolite concentration		
			ΣDEHP	0.17–0.71 μM	OR 1.44 (1.14, 1.82)*
			MEHP	NR	OR 1.13 (0.91, 1.4)
			MEHHP	NR	OR 1.51 (1.21, 1.88)*
			MEOHP	NR	OR 1.49 (1.19, 1.87)*
MECPP	NR	OR 1.36 (1.08, 1.73)*			
Stahlhut et al. 2007, Cross-sectional (United States)	1,451 adult males >18 years who were not taking insulin, oral hypoglycemic agents, or sex hormone agonists/antagonists; participants in NHANES 1999–2002.	Linear regression adjusted for age, age, race/ethnicity, total fat and calorie intake, physical activity level, smoking exposure, urinary Cr, GFR, ALT, and GGT	Association between ln-HOMA-IR and log-transformed urinary metabolite concentration		
			MEHP	11±1.3 μg/g Cr (mean±SE)	β 0.016 (NR)
			MEHHP	65.8±7.9 μg/g Cr	β 0.038 (NR)
			MEOHP	38.7±4.5 μg/g Cr	β 0.044 (NR)

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

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a	
Odds of incident type 2 diabetes					
Sun et al. 2014a, Cohort (United States)	Nurses with type-2 diabetes and matched controls from the NHS of female registered nurses aged 23–79 (394 cases, 393 controls) and the NHSII of female registered nurses aged 32–52 (577 cases, 577 controls); cases and controls were matched for age at urine collection, date and time of day of sample collection, ethnicity, fasting status when blood was drawn, and menopausal status and hormone replacement therapy at sample collection. Urine and samples collected from NHS participants aged 53–79 years during 2000–2002 and from NHSII participants aged 32–52 years during 1996–2001.	Multiple logistic regression adjusted for age at urine sample collection, ethnicity, fasting status, time of sample collection, menopausal status, postmenopausal hormone use (NHS only), smoking status, and use of hormone replacement therapy (NHSII only)	OR for incident type 2 diabetes comparing highest quartile of urinary metabolite concentration with lowest quartile (both studies combined)		
			ΣDEHP	NHS cases: 154.4–545.8 nmol/L NHS controls: 142.8–463.7 nmol/L NHSII cases: 201.4–586.3 nmol/L NHSII controls: 170.8–522.3 nmol/L	NR
			MEHP	NR	OR 0.97 (0.55, 1.69)
			MEHHP	NR	OR 1.54 (0.96, 2.46)
			MEOHP	NR	OR 1.42 (0.95, 2.11)
MECPP	NR	OR 2.17 (1.40, 3.38)*			
Significant ORs (2.05–2.30) were also observed for MECPP, but not other metabolites, in separate analyses of NHS and NHSII.					

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

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
Gestational diabetes and insulin resistance				
James-Todd et al. 2016a Cohort (United States [Boston])	298 pregnant women with full term births recruited from a prospective pregnancy cohort at Brigham and Women's Hospital that delivered between 2006 and 2008 (LIFECODES cohort), mean age 31.9 years, 47 with IGT; maternal urine samples collected at median 9.9 and 17.9 weeks of gestation. IGT was determined in the second or third trimester (median 26.1 weeks).	Multivariable logistic regression adjusted for age, race/ethnicity, education, baseline BMI, alcohol drinking, and smoking	Odds of having IGT (glucose levels >140 mg/dL) in the highest quartile of first trimester urinary metabolite concentration with the lowest quartile ΣDEHP Controls (n=251): 0.2–0.8 μmol/L (MEHP, IGT cases (n=47): 0.2–1.4 μmol/L MEHHP, (GM; SG-adj) MEOHP, MECPP)	OR 0.25 (0.08,0.85)* In other analyses, least geometric mean blood glucose levels were not associated with urine ΣDEHP levels (nonsignificant p for trend).
Shapiro et al. 2015, Cohort (Canada)	1,274 pregnant women (>18 years), members of birth cohort recruited during first trimester between 2008 and 2011 at 10 sites in 6 Canadian provinces (MIREC); n=47 cases of IGT and n=43 cases of GDM. Urine samples collected during the first trimester. IGT and GDM determined by chart review after delivery.	Multiple logistic regression adjusted for maternal age, race, pre-pregnancy BMI, education, and specific gravity	OR comparing the highest quartile of first-trimester urinary metabolite concentration with the lowest quartile ΣDEHP NR MEHP Controls: 2.6 (2.5) IGT cases: 2.3 (2.4) GDM cases: 2.7 (2.9) (GM [GSD]; SG-adj) MEHHP Controls: 10.6 (2.5) IGT cases: 10.4 (2.4) GDM cases: 11.4 (3.0) MEOHP Controls: 7.4 (2.3) IGT cases: 6.9 (2.2) GDM cases: 7.8 (2.7)	GDM OR 0.9 (0.3, 2.9) IGT OR 1.0 (0.3, 3.4) GDM or IGT OR 0.9 (0.4, 2.3) NR NR NR

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

Reference and study type	Population	Method of analysis	Urinary metabolite levels (IQR in ng/mL unless otherwise specified)	Effect estimate (95% CI) ^a
			No significant OR in any exposure quartile, and no significant trend across exposure quartiles.	
Robledo et al. 2015, Cohort (United States [Oklahoma])	72 pregnant women (18–38 years) without diabetes recruited during first prenatal care visit at the University of Oklahoma Medical Center Women's Clinic between February and June 2008. Oral glucose challenge test administered at median gestational age 26.3 weeks. Urine sample collected at enrollment.	Linear regression adjusted for urinary creatinine, gestational age at enrollment, and race/ethnicity	Difference between blood glucose levels (mg/dL) in highest versus lowest tertiles of urinary metabolite concentration	
			ΣDEHP 36.82–126.00	β -9.97 (-27.11, 7.17)
			MEHP 1.40–7.75	β 9.07 (-6.3, 24.45)
			MEHHP 10.35–40.85	β -5.55 (-21.77, 10.66)
			MEOHP 7.70–24.20	β -9.65 (-27.19, 7.89)
			MECPP 16.90–54.20	β -3.26 (-20.38, 13.86)

^aAsterisk indicates association with DEHP or DEHP metabolites; unless otherwise specified, values in parenthesis are 95% confidence limits.

ΣDEHP = sum DEHP metabolites; ALT = alanine aminotransferase; AUC = area under the curve; BMI = body mass index; CI = confidence interval; Cr = creatinine; DEHP = di(2-ethylhexyl)phthalate; ELEMENT = Early Life Exposure in Mexico to Environmental Toxicants; GDM = gestational diabetes mellitus; GFR = glomerular filtration rate; GGT = gamma-glutamyl transferase; GM = geometric mean; HOMA-IR = homeostatic model assessment-insulin resistance; IGT = impaired glucose tolerance; IQR = interquartile range; 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; min = minimum; MIREC = Maternal-Infant Research on Environmental Chemicals; NHANES = National Health and Nutrition Examination Survey; NHS = Nurses' Health Study; NHSII = Nurses' Health Study II; NR = not reported; OR = odds ratio; SD = standard deviation; SE = standard error; YOTA = Young Taiwanese Cohort

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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 within the Nurses' Health Study and Nurses' Health Study II (Sun et al. 2014a) 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 mono-2-ethyl-5-carboxypentylphthalate (MECPP) in urine (OR 2.17; 95% CI 1.40, 3.38). 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. 2016a).

Animal Studies. Glucose homeostasis may be impaired in animals following exposure to DEHP. Rajesh et al. (2013) reported altered glucose metabolism and homeostasis in Wistar rats exposed to doses ≥ 10 mg/kg/day via gavage for 30 days (lowest dose tested). Altered endpoints included decreased glycogen levels and glucose uptake in visceral adipose tissue, and elevated serum glucose levels. Oxidative stress and lipid peroxidation markers were also elevated in adipose tissue of treated rats from both groups. Gene expression analysis showed altered expression of insulin signaling molecules that could account for decreased glucose uptake into adipose tissue, and therefore increased serum glucose (Rajesh et al. 2013). However, no changes in serum glucose were observed in male Wistar rats exposed 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 F344 rats, increased serum glucose was observed in males exposed to doses ≥ 850.1 mg/kg/day for 13 weeks; 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). Serum glucose changes were not observed in 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 (Lin et al. 2011; Mangala Priya et al. 2014; Rajesh and Balsubramanian 2014a). In these studies, no

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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. Increases in visceral adipose tissue and adipocyte hypertrophy were observed in female mice 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, an appetite-controlling hormone, 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 pre-mating 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). Rajesh and Balasubramanian (2014a) 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 2014a). 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 $\geq 1,414$ 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.

Summary. A limited number of epidemiological studies found potential associations between DEHP exposure and diabetes-related outcomes (e.g., impaired glucose homeostasis) in humans (Attina and Trasande 2015; Huang et al. 2014a; James-Todd et al. 2012; Kim and Hong 2014; Kim et al. 2013; Lin et al. 2016; Trasande et al. 2013a). A limited number of animal studies report altered glucose homeostasis and metabolic syndrome.

2.19 CANCER

Epidemiological Studies—Cancer. Available epidemiological studies of the association between cancer and DEHP exposure in humans are limited to three case-control studies (Lopez-Carillo et al. 2010;

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Martinez-Nava et al. 2013; Holmes et al. 2014) in which exposure (as urinary metabolite levels) was measured after the outcome (breast cancer) was observed. 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, 2000) 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). 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 to 1,400 mg/kg/day for 102 weeks; however, only 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.

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; Melnick 2001; Rusyn and Corton 2012).

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As discussed in Section 2.9, DEHP activates PPAR α in rats and mice (Rusyn and Corton 2012). Therefore, it follows that observed liver tumors in rodents may be PPAR α -dependent. Key events identified in this mode of action are: (1) PPAR α 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 PPAR α -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 PPAR α -dependent tumors (Isenberg et al. 2000).

It is generally accepted that the PPAR α mode of action is not relevant to humans due to differences observed in key events downstream of PPAR α activation (Corton et al. 2018; Klaunig et al. 2003; Maloney and Waxman 1999). Guyton et al. (2009) reported that PPAR α activation may not be essential to rodent liver tumor formation since liver tumors have been observed in some studies using PPAR α -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 PPAR α -null mice; (3) comparison of findings in PPAR α -null mice to non-concurrent controls of a different strain; and (4) different molecular environments in PPAR α -null mice compared with wild-type mice (e.g., increased levels of background and DEHP-inducible levels of oxidative stress).

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 et al. 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 \geq 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

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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 to 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. 1952; 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).

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.

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Table 2-18. Genotoxicity of DEHP *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Prokaryotic organisms				
<i>Salmonella typhimurium</i> (TA98, TA100, TA1535, TA1538)	Gene mutation	–	–	Agarwal et al. 1985
<i>S. typhimurium</i> (NS)	Gene mutation	–	–	Astill et al. 1986
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	–	–	Kirby et al. 1983
<i>S. typhimurium</i> (TA100)	Gene mutation	–	+	Kozumbo et al. 1982
<i>S. typhimurium</i> (TA98)	Gene mutation	–	–	Sato et al. 1994
<i>S. typhimurium</i> (TA102)	Gene mutation	–	–	Schmezer et al. 1988
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	–	–	Simmon et al. 1977
<i>S. typhimurium</i> (TA100)	Gene mutation	–	–	Seed 1982
<i>S. typhimurium</i> (TA100)	Gene mutation	+	NS	Tomita et al. 1982b
<i>S. typhimurium</i> (TA98, TA100)	Gene mutation	–	–	Yoshikawa et al. 1983
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	Zeiger et al. 1985
<i>Escherichia coli</i> PQ37	Gene mutation	–	–	Sato et al. 1994
<i>E. coli</i> WP2UVRA ⁺	Gene mutation	–	–	Yoshikawa et al. 1983
<i>E. coli</i> WP2UVRA	Gene mutation	–	–	Yoshikawa et al. 1983
<i>S. typhimurium</i> (TA1535/psk 1002)	DNA damage	+	–	Okai and Higashi-Okai 2000
<i>Bacillus subtilis</i> (rec assay)	DNA damage	+	–	Tomita et al. 1982b
<i>S. typhimurium</i> (TA100)	Azaguanine resistance	–	–	Seed 1982
Eukaryotic organisms				
<i>Saccharomyces cerevisiae</i> (XV185-14C, D7, RM52, D6, D5, D6-1)	Gene mutation	–	–	Parry et al. 1985
<i>Saccharomyces cerevisiae</i> (JD1, D7-144, D7)	Gene conversion	–	–	Parry et al. 1985
<i>S. cerevisiae</i> (D61M, D6)	Mitotic aneuploidy	+	+	Parry et al. 1985
<i>S. cerevisiae</i> (D61M, D6)	Mitotic segregation	–	–	Parry et al. 1985
<i>Schizosaccharomyces pombe</i> (P1)	Gene mutation	–	–	Parry et al. 1985
<i>Aspergillus niger</i> (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

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Table 2-18. Genotoxicity of DEHP *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
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
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
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 leukocytes	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
SHE cells	Chromosomal aberrations	–	–	Tsutsui et al. 1993

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Table 2-18. Genotoxicity of DEHP *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
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
Human fetal pulmonary cells	Aneuploidy	–	NA	Stenchever et al. 1976
Rat RL4 liver cells	Polyploidy	–	NA	Priston and Dean 1985

^aMutagenic 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				
<i>Salmonella typhimurium</i> (TA98, TA100, TA1535, TA1538)	Gene mutation	–	–	Agarwal et al. 1985
<i>S. typhimurium</i> (NS)	Gene mutation	–	–	Astill et al. 1986
<i>S. typhimurium</i> (TA97, TA98, TA100, TA102)	Gene mutation	–	–	Dirven et al. 1991
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	–	–	Kirby et al. 1983
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	–	–	Ruddick et al. 1981

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Table 2-19. Genotoxicity of MEHP *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
<i>S. typhimurium</i> (TA100, TA102)	Gene mutation	-	-	Schmezer et al. 1988
<i>S. typhimurium</i> (TA100)	Gene mutation	-	±	Tomita et al. 1982b
<i>S. typhimurium</i> (TA98, TA100)	Gene mutation	-	-	Yoshikawa et al. 1983
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537)	Gene mutation	-	-	Zeiger et al. 1985
<i>Escherichia coli</i> (WP2 B/r)	Gene mutation	NS	± ^a	Tomita et al. 1982b
<i>E. coli</i> (WP2 <i>try</i> ⁻ [UvrA ⁺ and UvrA ⁻])	Gene mutation	-	-	Yoshikawa et al. 1983
<i>Bacillus subtilis</i> (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
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
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

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Table 2-19. Genotoxicity of MEHP *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
SHE cells	Chromosomal aberrations	+	-	Tsutsui et al. 1993
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

^aMutagenic 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 (<i>gpt</i> delta transgenic) (diet)	Gene mutation in liver	-	Kanki et al. 2005
Mouse (<i>lacZ</i> transgenic) (NS)	Gene mutation in liver	+	Boerrigter 2004
Mouse (<i>lacZ</i> 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
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 (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

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Table 2-20. Genotoxicity of DEHP *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
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
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-mediated assay			
<i>Salmonella typhimurium</i> (TA100); (rat host-mediated)	Gene mutation	–	Kozumbo et al. 1982
Eukaryotic organisms			
<i>Drosophila melanogaster</i> (feeding)	Mitotic recombination	–	Vogel and Nivard 1993
<i>D. melanogaster</i> (injection)	Sex linked recessive lethal	–	Yoon et al. 1985

– = negative result; + = positive result; DNA = deoxyribonucleic acid; *gpt* = guanine phosphoribosyltransferase; NS = not specified

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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, 27 *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 (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).

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). 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.

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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; 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. 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

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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*

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(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).