CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Human studies of DEHP provide primarily qualitative information on absorption and distribution and limited quantitative data on metabolite profiles and urinary excretion kinetics. DEHP toxicokinetics have been extensively studied in nonhuman primates (e.g., marmosets) and rodents, with most quantitative information derived from studies conducted in rats. An overview of these data is summarized below.

- At least 98% of inhaled radiolabeled DEHP is absorbed by the male rat. Based on volunteer studies, the expectation is that >70% of an oral dose is absorbed. Other experimental animals absorb a minimum of 30%. DEHP can be absorbed through skin. Approximately 2% of a dermal dose is absorbed in humans (6% in rats and 19–>50% in hairless guinea pigs).
- DEHP can saturate the enzymes responsible for metabolite absorption.
- No studies have been identified that provide reliable information about the distribution of DEHP in tissues (other than blood) in humans.
- DEHP has been detected in human adipose tissue collected at autopsy.
- Animal studies indicate that for all routes of exposure, the initial distribution is to liver, intestine, muscle, kidney, and fat (and lung during inhalation exposure).
- DEHP has been detected in placenta, amniotic fluid, fetal liver, and other fetal tissues in exposed rats. Mammary milk contains and transfers DEHP and MEHP to nursing rat pups.
- Tissue lipases hydrolyze DEHP. DEHP metabolites are further metabolized by cytochrome P450s, alcohol dehydrogenase, and aldehyde dehydrogenase.
- Most elimination of DEHP metabolites occurs by excretion in urine and feces (biliary secretion).
- Metabolite excretion profiles observed in humans are similar to those that have been observed in monkeys, rats, mice, hamsters, and guinea pigs, although species differences in relative abundance of metabolites and glucuronide conjugates have been reported.

3.1.1 Absorption

The uptake of particle-phase DEHP was studied in 16 volunteers exposed to 123±21 µg/m^3 full ring-deuterated DEHP (DEHP-D_4) for 3 hours (Andersen et al. 2018; Krais et al. 2018). DEHP uptake values of 0.51±0.34 µg/kg or 0.0014±0.00088 (µg/kg)/(µg/m^3)/hour were calculated from the urinary

concentrations of five DEHP metabolites. These values were adjusted for deposition, assuming that the deposited particles mass was 26% of the inhaled mass (determined by a multi-path particle dosimetry model). Absorption was also confirmed to occur through the lungs of humans as evidenced by identification of DEHP in the urine of infants exposed to DEHP during respiration therapy (Roth et al. 1988). Up to 98% of inhaled [^{14}C]-DEHP was recovered from urine, feces, and tissues of exposed male Sprague-Dawley rats (n=3) within 72 hours of exposure (Pegg 1982). Inhalation absorption of DEHP is also suggested by reported non-respiratory health effects observed following inhalation exposure (Table 2-1).

Oral absorption was demonstrated in four male volunteers (21–61 years old) who ingested a single dose (645±20 μg/kg) of DEHP-D_4 (Kessler et al. 2012). The concentration-time courses of DEHP-D_4, free MEHP-D_4, and total MEHP-D_4 in blood varied widely among the volunteers. Peak blood concentrations of DEHP-D_4 generally occurred 3–4 hours after dosing. Free and total MEHP-D_4 blood concentrations each exhibited two spikes at 3–4 and 5–10 hours after exposure. Mean area under the concentration-time course (AUC) values for 24 hours after dosing indicated that the blood burden of free MEHP-D_4 was 2-fold higher than the blood burden of DEHP-D_4. Total MEHP-D_4 in the blood consisted of 64% free MEHP-D_4 and 36% MEHP-D_4-β-glucuronide (Kessler et al. 2012). Measurement of DEHP urinary metabolites after ingestion of a single oral dose in humans (0.35, 2.15, or 48.5 mg) indicated that at least 70% of the oral dose was systemically absorbed (Koch et al. 2005a). Other human studies reported lower oral absorption (11–47%); however, these studies have methodological limitations, including analysis of a smaller number of urinary metabolites and use of unlabeled DEHP (Anderson et al. 2001; Koch et al. 2004; Schmid and Schlatter 1985). In all cases, the oral absorption is expected to be higher than reported due to the biliary excretion of orally absorbed DEHP, which is not accounted for in these studies.

Studies conducted in several different experimental animal models (cynomolgus monkey, marmoset, rats, mice, hamsters) have suggested that at least 30% of a single oral dose of ^{14}C administered as [^{14}C]-DEHP is systemically absorbed (Astill 1989; Astill et al. 1986; Daniel and Bratt 1974; Lake et al. 1984; Plichta et al. 2019; Rhodes et al. 1986; Short et al. 1987; Sjöberg et al. 1985a; Williams and Blanchfield 1974). In studies of dogs and rabbits, absorption was confirmed by the presence of phthalate in urine during 3 days postexposure (Shaffer et al. 1945). Absorption in rodents and monkeys has been underestimated because studies do not account for fecal excretion nor tissue storage of DEHP metabolites (Daniel and Bratt 1974; Rhodes et al. 1986).

In marmosets, 54–78% of a single oral dose of 100 mg/kg [^{14}C]-DEHP was excreted in urine and feces over 7 days (Kurata et al. 2012a). Oral absorption of DEHP appears to be lower in marmosets compared to rats based on blood and tissue levels of ^{14}C observed in the two species following oral dosing with [^{14}C]-DEHP (Kurata et al. 2012a; Rhodes et al. 1986) or measurements of plasma C_{max} and AUC at comparable doses (Kessler et al. 2004). Oral absorption of DEHP also appears to be greater in immature rats compared to mature rats. Plasma AUC for ^{14}C following a single oral dose of 1,000 mg/kg [^{14}C]-DEHP administered to rats at age 20 days was approximately twice that of rats that received the same dose at age 40 or 60 days (Sjöberg et al. 1985a). Plasma concentration data for 3- or 18-month-old marmosets, however, did not show an age-related change in oral absorption of radiolabel following administration of a single dose of 100 or 2,500 mg/kg [^{14}C]-DEHP (Kurata et al. 2012a). Plasma AUC data (all radiolabel) for 3-month-old marmosets suggest a saturation of absorption at higher doses (AUC/dose ratios were 0.374 and 0.108 for administered doses of 100 and 2,500 mg/kg, respectively) (Kurata et al. 2012a).

Hydrolysis of DEHP appears to be the rate-limiting step in the absorption of MEHP in the small intestine. In an *in vitro* preparation of rat small intestine, exposure of the intestinal mucosa to DEHP resulted in an absorptive flux of MEHP with no flux of DEHP, and MEHP was absorbed 7–8 times more rapidly when the intestinal mucosa was exposed to MEHP than when exposed to DEHP (White et al. 1980). Chang-Liao et al. (2013) estimated the bioavailability of DEHP following a single gavage dose of 100 mg/kg to be approximately 7% in male Sprague-Dawley rats based on comparison to a 10 mg/kg intravenous dose.

The appearance of DEHP in liver shortly after (e.g., 4 hours) an oral dose of DEHP has been used as an indirect measure of absorption of unhydrolyzed DEHP from the gastrointestinal tract (transport to the liver in the hepatic-portal blood). Gavage and intravenous studies have reported an apparent dose threshold for the appearance of DEHP in liver soon after dosing in rats and certain mouse strains (Albro 1986; Albro et al. 1982b). However, Astill (1989) found that no such absorption threshold existed when rats were fed DEHP in the diet at comparable doses and for prolonged feeding periods, indicating that the gavage and intravenous methods could influence absorption assessments. DEHP was not detected in the liver of rats 6 hours following intravenous administration of doses ≤500 mg/kg; however, over the dose range 500–1,000 mg/kg, DEHP concentration in the liver increased with increasing dose, suggesting an intravenous threshold for absorption of DEHP at approximately 500 mg/kg (Albro et al. 1982b). A similar dose-dependency in liver DEHP concentration was observed in CD-1 mice, with DEHP detected in the liver following gavage doses in excess of approximately 500 mg/kg (Albro 1986). No threshold for DEHP absorption was detected in B6C3F1 mice following oral doses of ranging from 20 to 575 mg/kg, as

indicated by liver DEHP concentrations (Albro 1986). The observations of apparent thresholds for DEHP gavage absorption are consistent with either exposure methodology effects or saturation of DEHP hydrolysis in the gastrointestinal tract, leading to increased absorption of unhydrolyzed DEHP. *In vitro* studies have shown that hydrolysis of DEHP to MEHP in contents of rat caecum and small intestine is saturable (Rowland 1974). Albro and Thomas (1973) suggested that there is little chance that DEHP would be absorbed as an intact molecule following oral exposure.

DEHP applied dermally penetrates skin and can be absorbed into the systemic circulation (Chu et al. 1996; Deisinger et al. 1998; Elsisi et al. 1989; Wester et al. 1998). Wester et al. (1998) observed, in humans, that approximately 1% of a ^{14}C dose applied as [^{14}C]-DEHP (18.5 µg/cm^2 dissolved in ethanol) was excreted in urine in 7 days. The dose was applied to the forearm of five to six adults and washed 24 hours after application. The urinary excretion of [^{14}C]-DEHP was also measured following intravenous injection in Rhesus monkeys to account for fecal excretion and tissue storage. From these data, Wester et al. (1998) estimated the total human dermal dose absorbed to be 1.8±0.5%. In rats, approximately 6% of an applied dose of [^{14}C]-DEHP (5–8 mg/cm^2, dissolved in ethanol) was excreted (urine plus feces) in 7 days (Elsisi et al. 1989). The dose was applied to the shaved back, covered with a perforated plastic bandage, and left in place for 7 days. Absorption, as measured by ^{14}C in excreta and carcass, was much lower in rats when the DEHP dose was applied as a polyvinyl carbonate film containing [^{14}C]-DEHP (Deisinger et al. 1998). A 24-hour exposure to approximately 400 mg DEHP resulted in 0.01% of the applied dose appearing in the excreta (urine plus feces) and carcass after 7 days (Deisinger et al. 1998).

Dermal absorption of DEHP was higher in hairless guinea pigs than in rats (Chu et al. 1996; Ng et al. 1992). A dermal dose (13 µg/cm^2) of [^{14}C]-DEHP (dissolved in acetone, applied to the back, covered with a non-occlusive bandage, and left in place for 24 hours) resulted in excretion (urine plus feces) of approximately 21% of the applied dose in hairless guinea pigs (Ng et al. 1992). The estimated dermal absorption was approximately 53% of the applied dose (calculated from the cumulative 7-day excretion of ^{14}C following a single intramuscular dose of [^{14}C]-DEHP).

Chu et al. (1996) applied a 442 µg/cm^2 (dissolved in acetone) dose of radiolabeled DEHP to the backs of hairless guinea pigs. A non-occlusive bandage covered the application site and for 7 days, and feces and urine were collected. Chu et al. (1996) determined that 19% of the applied dose was dermally absorbed and either excreted or stored within the body.

In vitro studies have provided estimates of transdermal flux rates of ^{14}C when [^{14}C]-DEHP is applied to the epidermal surface (Barber et al. 1992; Hopf et al. 2014; Ng et al. 1992; Scott et al. 1987; Wester et al. 1998). Experiments using fresh dermatomed human abdominal skin demonstrated that an aqueous solution of DEHP-D_4 readily permeated the skin (K_p of $15.1x10^{-5}$ cm/hour), while the permeability of neat DEHP was much lower (K_p of $0.13x10^{-5}$ cm/hour) (Hopf et al. 2014). Two studies have measured and compared permeability coefficients for ^{14}C in skin preparations from humans and rats exposed to [^{14}C]-DEHP; both studies found human skin to be approximately 4-fold more permeable than rat skin (Barber et al. 1992; Scott et al. 1987). Barber et al. (1992) estimated permeability coefficients to be $1.05±0.21x10^{-7}$ cm/hour for isolated human epidermal membranes and $4.31±1.34x10^{-7}$ cm/hour for isolated rat skin (whole skin). Scott et al. (1987) estimated coefficients to be $0.57±0.12x10^{-5}$ cm/hour for human epidermal membranes and $2.28±0.23x10^{-5}$ cm/hour for rat epidermis.

In vitro studies have also been conducted with preparations of hairless guinea pig skin and in perfused pig skin flaps (Ng et al. 1992; Wester et al. 1998). These studies did not derive permeability coefficients; however, they do provide ^{14}C flux rates for similar initial doses applied to the epidermal surfaces. The flux rate in the perfused pig skin was approximately 10-fold lower; 0.003 µg/cm^2/hour at a starting dose of 18.5 µg/cm^2 (Wester et al. 1998) in the pig epidermal membranes, compared to 0.035 µg/cm^2/hour at a starting dose of 14 µg/cm^2 in the guinea pig skin (Ng et al. 1992). In the Ng et al. (1992) study, ^{14}C recovered in the receptor fluid was analyzed to determine whether the ^{14}C that was transferred across the skin preparation was [^{14}C]-DEHP or [^{14}C]-MEHP. Approximately 70% of the transdermal flux of ^{14}C across the hairless guinea pig skin was attributed to MEHP. Treatment of the preparation with an esterase inhibitor (phenylmethylsulfonyl fluoride) decreased the MEHP contribution to the flux rate from 70 to 45%; however, total ^{14}C flux was not significantly affected (3.36±0.37%/24 hours versus 2.67±0.42%/24 hours). These results suggest that, while hydrolysis of DEHP to MEHP occurred in the skin, it was not a rate-limiting step for *in vitro* dermal absorption.

3.1.2 Distribution

No studies were identified that provide reliable information about the distribution of DEHP in tissues (other than blood) in humans. While DEHP has been detected in human adipose tissues collected at autopsy (Mes et al. 1974), contamination from plastics used in the handling and storage of the tissues may have contributed to the levels of DEHP detected in this study.

More direct measurements of tissue distribution are available from studies conducted in animals that received doses of labeled DEHP (e.g., [^{14}C]-DEHP). The tissue distribution of ^{14}C following intravenous, oral, inhalation, and dermal dosing with [^{14}C]-DEHP has been studied in rodents, dogs, pigs, and nonhuman primates (Ikeda et al. 1980; Kurata et al. 2012a; Pegg 1982; Rhodes et al. 1986; Tanaka et al. 1975). In general, for all of the above routes of exposure, the initial distribution (within 4 hours of dosing) is dominated by uptake of ^{14}C in liver, intestine, muscle, kidney, and fat (and in lung during inhalation exposure) (Pegg 1982). Concentrations in liver, spleen, intestine, lung, kidney, heart, and adipose can exceed that of blood (Rhodes et al. 1986; Tanaka et al. 1975). Distribution to the intestine occurs following intravenous dosing, indicating transport of absorbed ^{14}C to the intestine (Tanaka et al. 1975; Wallin et al. 1974). The elimination from fat is slower than from other tissues and, as a result, the contribution of fat to ^{14}C body burden increases over time following a single dose of [^{14}C]-DEHP, as ^{14}C is eliminated from other tissues (Ikeda et al. 1980; Tanaka et al. 1975). In male Sprague-Dawley rats exposed to an aerosol (0.24–0.61 μm particle size range) of [^{14}C]-DEHP (83 mg/m^3) for 6 hours, approximately 50% of the inhaled ^{14}C was excreted in urine, 40% was excreted in feces within 72 hours, and approximately 5–7% remained in the carcass (Pegg 1982).

Although numerous studies have measured tissue levels of ^{14}C following dosing with [^{14}C]-DEHP, Tanaka et al. (1975) reported time-course observations for ^{14}C in various tissues (male Wistar rats) following a single intravenous or oral dose of [^{14}C]-DEHP. Tissue ^{14}C levels were expressed as percent of dose and as dose-adjusted tissue ^{14}C concentrations. The latter metric allows comparisons of tissue ^{14}C concentrations and tissue ^{14}C kinetics for the two exposure routes (Tables 3-1 and 3-2). Following an intravenous dose (50 mg/kg), the highest concentrations of ^{14}C were observed in liver, and tissue:blood concentration ratios 1 hour following the intravenous dose were >1 for liver (53), spleen (20), intestine (tissue and contents, 7.8), lung (4.7), kidney (3.0), and heart (1.9). Seven days following the intravenous dose, the total body burden of ^{14}C was <1% of the administered dose and the highest ^{14}C concentration was in adipose. Tissue:blood concentration ratios were ≥1 in adipose (7.5), lung (2.2), liver (2.0), kidney (1.5), and intestine (1.1). A similar pattern of distribution was observed following the oral dose of ^{14}C-DEHP (500 mg/kg) (Tanaka et al. 1975). The highest concentrations (excluding the gastrointestinal tract) were observed in liver 3 hours following the oral dose. At that time, tissue:blood concentrations were ≥1 in liver (6.9), kidney (4.8), lung (2.8), spleen (2.4), heart (1.8), and muscle (1.2). Twenty-four hours following the oral dose, the body burden of ^{14}C (excluding the gastrointestinal tract) was <3% of the administered dose.

Table 3-1. Tissue Distribution of ^{14}C Following an Intravenous Dose of 50 mg/kg [^{14}C]-DEHP in Male Wistar Rats[a]

	Time following dose (hours)						
Tissue	1	2	3	6	12	24	168
Liver	15	12	10	7.3	5.6	1.5	0.04
Spleen	5.7	2.1	0.63	0.4	3.8	0.4	0.015
Intestine	2.2	3.0	3.7	3.7	1.7	1.9	0.022
Lung	1.3	0.76	0.64	0.47	0.23	0.07	0.045
Kidney	0.83	0.48	0.54	0.43	0.18	0.12	0.03
Heart	0.54	0.45	0.38	0.33	0.18	0.06	0.015
Blood	0.28	0.16	0.19	0.15	0.09	0.08	0.02
Adipose	0.25	0.20	0.09	0.10	0.21	0.18	0.15
Stomach	0.15	0.16	0.13	0.25	0.14	0.07	0.015
Muscle	0.12	0.13	0.13	0.15	0.07	0.22	0.015
Testicle	0.035	0.030	0.028	0.036	0.026	0.011	0.005
Brain	0.020	0.026	0.031	0.028	0.034	0.012	0.006

[a]Values are ^{14}C activity (dpm) per g tissue per dose/kg body weight (dpm/g per mg/kg).

Source: Tanaka et al. 1975

Table 3-2. Tissue Distribution of ^{14}C Following an Oral Dose of 500 mg/kg [^{14}C]-DEHP in Male Wistar Rats[a]

	Time following dose (hours)					
Tissue	1	2	3	6	12	24
Stomach	33	17	8.1	5.3	1.4	0.29
Intestine	3.7	5.5	6.5	3.6	5.7	6.9
Liver	0.43	0.44	0.69	0.66	0.36	0.18
Kidney	0.42	0.36	0.48	0.61	0.32	0.090
Lung	0.10	0.32	0.28	0.23	0.13	0.020
Spleen	0.070	0.12	0.24	0.13	0.030	0.0060
Heart	0.096	0.14	0.19	0.27	0.11	0.030
Muscle	0.080	0.10	0.12	0.11	0.04	0.008
Blood	0.060	0.07	0.10	0.11	0.06	0.030
Testicle	0.020	0.05	0.09	0.09	0.03	0.006
Adipose	0.42	0.10	0.08	0.11	0.05	0.020
Brain	0.010	0.025	0.036	0.018	0.05	0.00030

[a]Values are ^{14}C activity (dpm) per g tissue per dose/kg body weight (dpm/g per mg/kg).

Source: Tanaka et al. 1975

Following oral doses of [^{14}C]-DEHP administered to pregnant rats, ^{14}C has been detected in placenta, amniotic fluid, and fetal liver and other fetal tissues (Calafat et al. 2006; Clewell et al. 2010; Singh et al.

1975; Stroheker et al. 2006). Plasma DEHP and MEHP kinetics have been compared in pregnant and nonpregnant rats and marmosets. These studies indicate that plasma C_{max} and dose-adjusted plasma AUC are not markedly affected by pregnancy in these species (Kessler et al. 2004). The amniotic fluid:maternal plasma concentration ratio was approximately 0.2–0.3 following oral doses (750 mg/kg/day) administered to rats on GDs 14–21 (Stroheker et al. 2006). A major fraction of the ^{14}C that is transferred to the fetus appears in the liver. Liver ^{14}C was approximately 30% of total fetal ^{14}C burden following an oral dose of [^{14}C]-DEHP (750 mg/kg) administered on GDs 14–21 (Stroheker et al. 2006). When dosing was extended to PND 4, ^{14}C was detected in the livers of pups (3–5% of pup ^{14}C burden). Lactational exposure, as well as residual ^{14}C from *in utero* exposure, could have contributed to the ^{14}C observed in the pups. Kurata et al. (2012a) compared the distribution of ^{14}C in fetal blood, liver, kidney, and testes 24 hours after administration of a single gavage dose of 100 mg/kg [^{14}C]-DEHP on GD 20 in rats and GD 130 in marmosets. Radioactivity was highest in all tissues of fetal rats compared to fetal marmosets. MEHP was detected in the livers of mouse offspring (fetuses and PND 2 pups) following DEHP administration in the diet (0.01 and 0.05%) of pregnant dams (dosed throughout gestation) (Hayashi et al. 2012). DEHP lipase activity and MEHP concentrations were higher in pregnant dams compared to postpartum dams or nonpregnant mice.

DEHP and MEHP transfer to mammary milk. Milk concentrations of DEHP and MEHP were approximately 216 and 25 µg/mL, respectively, following oral doses of DEHP (2,000 mg/kg) administered to rats on days 15–17 of lactation (Dostal et al. 1987). Milk:maternal plasma concentration ratios in this study were >200 for DEHP and 0.3 for MEHP. DEHP and MEHP were not detected in pup plasma, which may reflect low bioavailability of DEHP and MEHP from milk, or rapid clearance of DEHP and MEHP from the pup plasma (the pups were analyzed 3–4 hours after the last maternal dose). DEHP was detected in livers of rat pups that nursed from dams that received oral doses of DEHP (2,000 mg/kg/day) from PND 1 through 21, indicating that DEHP in milk is bioavailable (Parmar et al. 1985). Supporting this are studies in which pups received oral doses of [^{14}C]-DEHP (in lipid emulsion). Liver ^{14}C was approximately 27% of the administered oral dose 24 hours following an oral dose of DEHP (0.7 mg/kg) administered on PND 3. Liver levels decreased to approximately 8% of the dose when administered on PND 10 and approximately 2% of the dose when administered on PND 20 (Eriksson and Darnerud 1985).

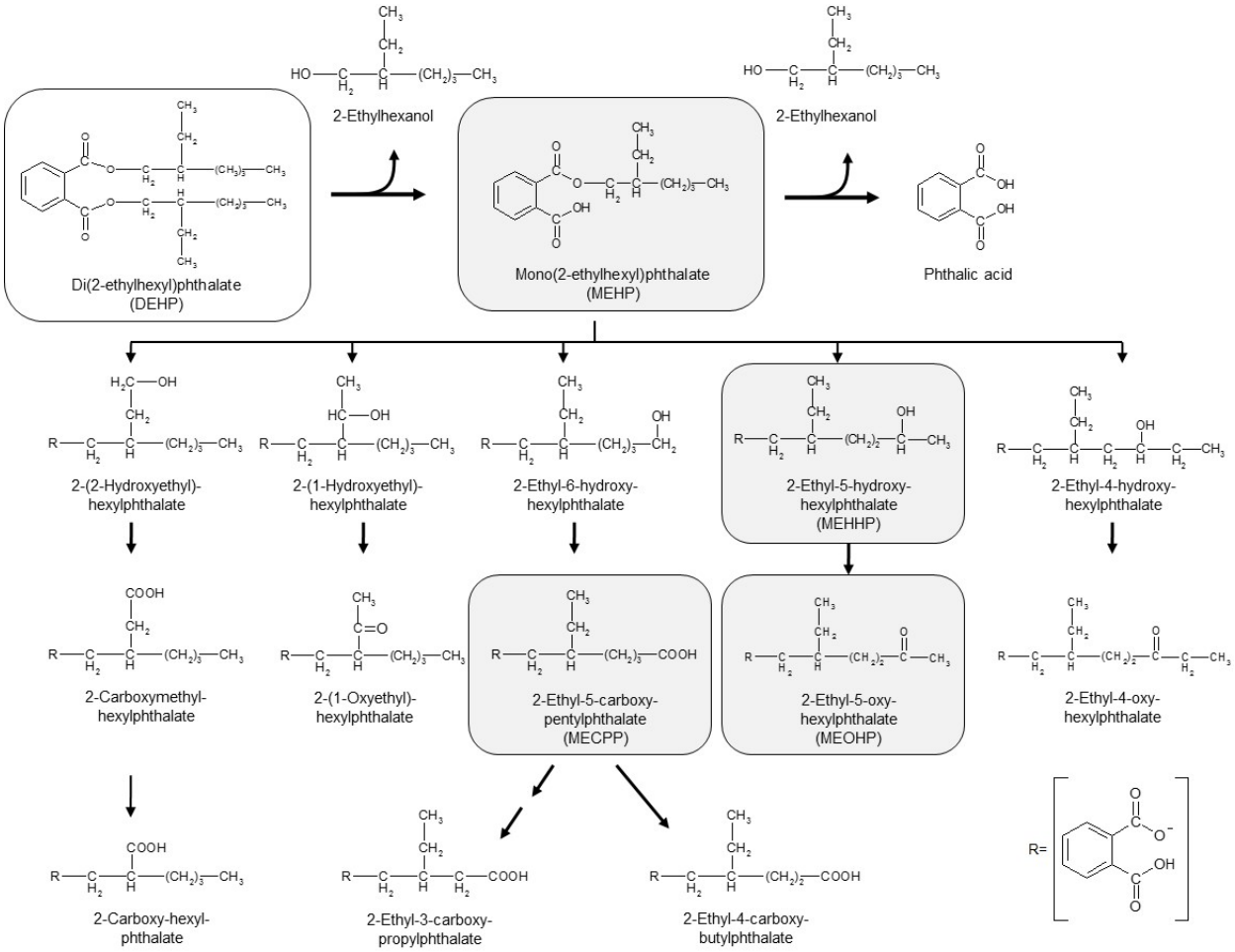
3.1.3 Metabolism

The metabolism of DEHP has been studied in humans and various animal models, including nonhuman primates and rodents (Albro 1986; Albro et al. 1981, 1982a, 1982b, 1983; Anderson et al. 2011; Astill 1989; Choi et al. 2012, 2013; Hayashi et al. 2012; Ito et al. 2014; Koch et al. 2005a, 2005b; Kurata et al. 2012a, 2012b; Lhuguenot et al. 1985; Schmid and Schlatter 1985; Silva et al. 2006). Figure 3-1 depicts the metabolic pathways for DEHP.

The first step in the metabolism of DEHP is hydrolytic cleavage to yield MEHP and 2-EH. The hydrolysis reaction is catalyzed by "DEHP hydrolases," which may include several different carboxyesterases, including lipases. DEHP hydrolase activity has been detected in a variety of tissues including pancreas, intestinal mucosa, liver, kidneys, lungs, skin, testes, and plasma (Albro 1986; Choi et al. 2012; Hopf et al. 2014; Ozaki et al. 2017). The pancreatic tissue is the richest source of DEHP hydrolase activity, whereas adipose has a relatively low activity. Pancreatic lipases secreted into the small intestine contribute DEHP hydrolase activity to the intestinal contents (White et al. 1980). This activity, along with esterases in the intestinal mucosa, results in substantial hydrolysis of ingested DEHP (to MEHP) at the gastrointestinal portal of entry (Barber et al. 1994; Rowland 1974; Rowland et al. 1977). Enzymes in gut microflora and gut contents can also convert DEHP to MEHP before absorption occurs (Rowland et al. 1977). Hydrolysis of DEHP in the gastrointestinal tract is saturable (Albro 1986; Albro et al. 1982b; Rowland 1974). This contributes to a dose-dependence in the bioavailability of DEHP, with increasing bioavailability of DEHP as dose approaches the saturating level in the gastrointestinal tract.

Although absorption of DEHP occurred in rats following oral doses >500 mg/kg (Albro et al. 1982a), DEHP was not detected in plasma following oral DEHP doses of 500–1,000 mg/kg/day for 7 days in rats (Sjöberg et al. 1986). These studies suggest that esterase activity in plasma, liver, and other tissues was sufficient to completely hydrolyze absorbed DEHP before it appears in plasma, even after oral doses of DEHP that would saturate hydrolysis in the gastrointestinal tract. Pollack et al. (1985a) estimated that approximately 80% of a 2,000 mg/kg oral dose of [^{14}C]-DEHP had been hydrolyzed prior to the appearance of ^{14}C in plasma in rats. Other studies conducted in rats and marmosets have shown that following an oral dose of DEHP, most of the phthalate that appears in plasma is MEHP and not DEHP (Kessler et al. 2004; Koo and Lee 2007). These studies suggest that as a result of the rapid hydrolysis of DEHP during and following absorption; the ^{14}C in plasma primarily reflects that of MEHP and MEHP metabolites rather than DEHP.

Figure 3-1. Metabolic Pathway of DEHP*



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

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

Species differences in DEHP hydrolase activity have been reported. Ito et al. (2005) compared activities in tissues (kidney, liver, lung, and small intestine) of mice, rats, and marmosets. The highest activities were observed in mice and the lowest activities were observed in marmosets. DEHP hydrolase observed in marmoset liver homogenates was approximately 5–10% of that of the mouse and rat. Ito et al. (2005) also measured the K_m and V_{max} for DEHP hydrolase activity in liver microsomes, a source of lipase and DEHP hydrolase activity (Table 3-3). Relative to rats and mice, marmosets had a higher K_m and lower V_{max}, with a V_{max}/K_m ratio that was <1% of that of rats and mice (i.e., lower intrinsic clearance). Relatively low activities of DEHP hydrolase in marmosets may at least partially explain the lower oral bioavailability of DEHP metabolites in marmosets compared to rats—see further discussion in Section 3.1.1. Ito et al. (2014) compared DEHP hydrolase activity in liver microsomes from mice and 38 human subjects (liver samples obtained from deceased donors). Mean DEHP hydrolase activity in human liver microsomes was 5-fold lower than the activity measured using mouse microsomes. Similar to marmosets, human hydrolase kinetics were characterized by a higher K_m and a lower V_{max} than mice, resulting in a 6.7-fold lower V_{max}/K_m ratio (Ito et al. 2014; Table 3-3). The inter-individual variation in DEHP hydrolase activity was approximately 10-fold among the 38 donors (primarily Caucasian males between the ages of 16 and 80 years). Hanioka et al. (2019) examined the kinetics of DEHP hydrolysis by liver and intestinal microsomes from humans, monkeys, dogs, rats, and mice. For liver microsomes, K_m values were similar among species, while V_{max} values varied up to 9-fold. Intrinsic clearance values (V_{max}/K_m) followed the order of mice > dogs > monkeys $\geq$ rats > humans. V_{max} and intrinsic clearance values were 5–25% lower for intestinal microsomes (compared with liver microsomes) from mice, rats, and monkeys, and DEHP hydrolysis activity was not detected in dog or human intestinal microsomes.

Table 3-3. Michaelis-Menten Constants for DEHP Hydrolase Activity in Liver Microsomes[a]

	Ito et al. (2005)			Ito et al. (2014)	
Reaction parameters	Mouse	Rat	Marmoset	Mouse	Human
K_m (mmol/L)	0.012	0.006	1.357	0.0076	0.0144
V_{max} (nmol/minute/mg protein)	3.91	1.32	0.49	5.45	1.52
V_{max}/K_m ratio	333	227	1.38	714	106

[a]Values represent the mean of triplicate analyses for each group.

Sources: Ito et al. 2005, 2014

Hydrolysis of the second ester bond of DEHP to convert MEHP to phthalic acid is a relatively minor pathway. The major pathways of metabolism of MEHP are ω- and ω-1-oxidation of the aliphatic side

chain, which forms side-chain hydroxyl products, followed by α- or β-oxidation and formation of side-chain carboxylic acid and ketone products. The ω- and ω-1-oxidation reactions are mediated by CYP isozymes, specifically human recombinant CYP2C9*1, CYP2C9*2, and CYP2C19 and rat recombinant CYP2C6 (Choi et al. 2012, 2013). Secondary α- or β-oxidation reactions have been attributed to alcohol dehydrogenase or aldehyde dehydrogenase (Albro and Lavenhar 1989; Ito et al. 2005). The oxidized phthalate metabolites of MEHP can be conjugated with glucuronic acid to form acyl-glucuronides (Albro 1986; Astill 1989; Silva et al. 2003; Sjöberg et al. 1991). Conjugation of MEHP and MEHP metabolites with glucose to form β-glucosides has also been detected in mouse urine; however, it appears to be a minor conjugation pathway relative to the glucuronide pathway (Egestad and Sjöberg 1992; Egestad et al. 1996). No other conjugation products of DEHP metabolites have been detected (e.g., sulfate, glutathione, taurine). Metabolites of the aromatic moiety of DEHP have not been reported. The 2-EH product of hydrolysis of DEHP is metabolized through oxidative pathways that include 2-ethylhexanoic acid keto acid derivatives, which appear to be products of β-oxidation (Albro and Corbett 1978).

The primary urinary metabolites of DEHP in humans include MEHP, MEHHP, 2-ethyl-5-oxyhexyl-phthalate; MEOHP, MECPP, and the corresponding acyl-glucuronides (Albro et al. 1982a; Anderson et al. 2011; Ito et al. 2014; Koch et al. 2005a, 2005b; Kurata et al. 2012a; Schmid and Schlatter 1985; Zhao et al. 2018). Metabolite excretion profiles observed in humans are similar to those that have been observed in monkeys, rats, mice, hamsters, and guinea pigs, although species differences in relative abundance of metabolites and glucuronide conjugates have been reported (Albro et al. 1981, 1982a, 1982b; Astill 1989; Kurata et al. 2012a, 2012b; Lhuguenot et al. 1985, 1988; Rhodes et al. 1986; Short et al. 1987). Relative abundances of DEHP metabolites excreted in urine of various animal species are presented in Table 3-4 (based on Albro et al. 1982a). Guinea pigs excreted relatively few MEHP oxidation products, suggesting low rates of oxidative metabolism of MEHP in this species. By contrast, rats excreted MEHP oxidation products but only trace levels of MEHP, indicating extensive oxidative metabolism of MEHP in this species. Species differences in conjugation patterns have also been observed. Phthalate metabolites of DEHP were excreted predominantly as glucuronide conjugates in humans and in monkeys, whereas glucuronide conjugates were not observed in rats (Albro et al. 1982a). Based on studies in which urine was treated with aryl sulfatase, acylase I, and carboxypeptidase A, conjugation of DEHP metabolites with glutathione, sulfates, or amino acids (e.g., taurine) does not occur in rats, mice, guinea pigs, or hamsters (Albro et al. 1982a). More recent studies confirm that urinary metabolites of DEHP are highly conjugated to glucuronide in humans and marmosets compared to rats (Kurata et al. 2012a, 2012b). Zhao et al. (2018) demonstrated that the relative proportion of the primary urinary metabolites of DEHP in pregnant women varies with the stage of pregnancy and maternal age.

Table 3-4. Comparison of Phthalate Metabolites in Urine Following Dosing with DEHP

	Percentage of total metabolites in urine[a]					
Metabolite	Rat	Mouse	Guinea pig	Green monkey	Man	Hamster
Residual DEHP	–	0.5	–	2.2	–	0.3
MEHP	Trace	18.6	71.2	28.9	18.3	4.5
MECPP	51.3	1.1	6.9	4.2	5.3	14.0
MEOHP	2.6	14.9	1.1	5.9	12.1	10.2
MEHPP	13.3	12.3	3.4	38.2	36.2	32.7
Free[b]	100[c]	36[d]	35	20	20	85
Conjugated[b]	0[d]	64[d]	65	80	80	15

[a]Urine containing 90% of administered ^{14}C following a single oral (rat, mouse, guinea pig, hamster) or intravenous (monkey, human) dose of [^{14}C]-DEHP were pooled. Data for rat, mouse, guinea pig, and hamster represent pooled urines from three animals; data for monkeys and humans represent two pooled urine samples.
[b]Percent of total ^{14}C not conjugated or conjugated with glucuronic acid (based on comparisons of urine treated or not treated with β-glucuronidase).
[c]Three rat strains.
[d]CD strain.

MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHP = mono(2-ethylhexyl)phthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEOHP = mono-2-ethyl-5-oxyhexylphthalate

Source: Albro et al. 1982a

3.1.4 Excretion

DEHP is mostly metabolized to MEHP and other DEHP metabolites. Elimination of these metabolites occurs by excretion in urine and feces (Daniel and Bratt 1974; Koch et al. 2004, 2005a; Kurata et al. 2012a, 2012b; Shaffer et al. 1945). Studies conducted in several different experimental animal models (Cynomolgus monkey, marmoset, rats, mice, hamsters) have shown that approximately 30–50% of a single oral dose of ^{14}C administered as [^{14}C]-DEHP is excreted in urine (Astill 1989; Astill et al. 1986; Daniel and Bratt 1974; Lake et al. 1984; Rhodes et al. 1986; Short et al. 1987; Sjöberg et al. 1985a; Williams and Blanchfield 1974). Doses utilized in these studies ranged from 85 to 2,000 mg/kg. Urinary excretion by humans was reported to be greatest 5-7 hours after exposure, totaling 4.5% in 24 hours (Shaffer et al. 1945). Excretion was similar in dogs, being greatest on day 2 post-exposure and totaling 2.0 or 4.5% in 3 days. Significantly greater excretion ranging from 26 to 65% in 3 days was reported for rabbits (Shaffer et al. 1945). DEHP and MEHP were detected by high-performance liquid chromatography (HPLC) in rat urine following doses of 40 to 1,000 mg/kg DEHP (Koo and Lee 2007); however, DEHP was not detected by ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) in urine from rats exposed to 100 mg/kg (Chang-Liao et al. 2013). DEHP

was not detected in human urine following single oral doses of DEHP-D_4 (3 mg or ~0.04 mg/kg from Kurata et al. [2012a]; 0.005–0.65 mg/kg from Koch et al. [2004, 2005a]). MEHP has also been detected in human sweat, which suggests that perspiration may also contribute to the elimination of DEHP (Genuis et al. 2012).

Fecal excretion results from biliary secretion of DEHP metabolites. [^{14}C]-MEHP, but not [^{14}C]-DEHP, was detected in bile of rats following an oral dose of [^{14}C]-DEHP (2.6 mg/kg) (Daniel and Bratt 1974). Metabolites delivered into the small intestine from biliary secretion may be reabsorbed, resulting in an enterohepatic circulation of DEHP-derived phthalates (Keys et al. 1999). Following oral or intravascular dosing with DEHP, serum concentrations of MEHP exhibit an oscillation in some reports that has been interpreted as indirect evidence for enterohepatic circulation (Kessler et al. 2004; Ljungvall et al. 2004; Pollack et al. 1985a; Sjöberg et al. 1985b); however, such a pattern was not observed in rats orally exposed to 100 mg/kg (Chang-Liao et al. 2013). Enterohepatic circulation is discussed further in context with physiologically-based toxicokinetic models of DEHP (Section 3.1.5).

Estimates of the relative contribution of the urinary and biliary routes vary widely. Estimates of urinary excretion following an oral dose of isotopically-labeled DEHP in humans range from 11 to 74% (Anderson et al. 2001; Koch et al. 2004, 2005a; Schmid and Schlatter 1985). Daniel and Bratt (1974) measured urinary and biliary ^{14}C following an oral dose of [^{14}C]-DEHP (2.6 mg/kg) in rats and estimated the urinary:biliary excretion ratio to be approximately 3:1. Other studies conducted in animals found urinary:fecal excretion ratios to be 2:1 in marmosets following an intravenous dose of 100 mg/kg DEHP (Rhodes et al. 1986), approximately 1–3:1 in rats following a dermal dose (Deisinger et al. 1998), and 4–5:1 in hairless guinea pigs following a dermal dose (Ng et al. 1992). The urinary:fecal excretion ratio in marmosets given a single oral dose of [^{14}C]-DEHP (100 or 2,500 mg/kg) was approximately 1:2–5 (cumulative excretion over 7 days postdosing) (Kurata et al. 2012a).

Elimination half-life ($t_{1/2}$) values for DEHP and MEHP have been estimated in humans, marmosets, pigs, and rats. Estimates of the blood, serum, or plasma elimination $t_{1/2}$ for MEHP following exposure to DEHP range from 2 to 4 hours in humans and marmosets and from 1.1 to 9.4 hours in rats (Table 3-5) (Kessler et al. 2004, 2012; Koch et al. 2004, 2005a; Koo and Lee 2007; Ljungvall et al. 2004; Oishi 1989, 1990; Pollack et al. 1985a; Sjöberg et al. 1985b; Teirlynck and Belpaire 1985). After DEHP administration in rats, the range of elimination values for DEHP from blood or plasma is wider than observed for MEHP, with reported values for $t_{1/2}$ ranging from 0.5 to 19 hours (Chang-Liao et al. 2013; Kessler et al. 2004; Koo and Lee 2007; Oishi 1989, 1990; Pollack et al. 1985a; Sjöberg et al. 1985b).

Table 3-5. Blood, Serum, or Plasma Elimination Half-Lives ($t_{1/2}$) for DEHP and MEHP

Species	Route of administration[a]	Dose (mg/kg)	Measured chemical	Measured medium	Elimination $t_{1/2}$ (hour)	Clearance (mL/hour/kg)	Reference
After administration of DEHP							
Human	Oral	0.645	DEHP	Blood	4.3	NA	Kessler et al. 2012
Human	Oral	0.645	MEHP	Blood	1.9 and 4.4 (biphasic[c])	NA	Kessler et al. 2012
Human	Oral	0.65	MEHP	Serum	2.0	NA	Koch et al. 2004, 2005a
Marmoset	Oral	30	MEHP	Blood	2.2[d]	NA	Kessler et al. 2004
Rat	Oral	1,000	DEHP	Blood	3.3	NA	Kessler et al. 2004
Rat	Oral	1,000	DEHP	Blood	17	NA	Oishi 1989
Rat	Oral	2,000	DEHP	Blood	16	NA	Pollack et al. 1985a
Rat	Oral	30	MEHP	Blood	2.8[d]	NA	Kessler et al. 2004
Rat	Oral	500	MEHP	Blood	3.1[d]	NA	Kessler et al. 2004
Rat	Oral	1,000	MEHP	Blood	3.9[d]	NA	Kessler et al. 2004
Rat	Oral	1,000	MEHP	Blood	5.8	NA	Oishi 1989
Rat	Oral	2,000	MEHP	Blood	6.7	NA	Pollack et al. 1985a
Rat	Oral	2,000	MEHP	Blood	7.4	NA	Oishi 1990
Rat	Oral	500	[$^{14}CO_2$][e]	Blood	11[d]	NA	Tanaka et al. 1975
Rat	Oral	40	DEHP	Plasma	19	552	Koo and Lee 2007
Rat	Oral	100	DEHP	Plasma	0.5	NA	Chang-Liao et al. 2013
Rat	Oral	200	DEHP	Plasma	15	2,116	Koo and Lee 2007
Rat	Oral	400	DEHP	Plasma	ND	NA	Teirlynck and Belpaire 1985
Rat	Oral	1,000	DEHP	Plasma	13	5,493	Koo and Lee 2007
Rat	Oral	2,800	DEHP	Plasma	ND	NA	Teirlynck and Belpaire 1985
Rat	Oral	40	MEHP	Plasma	9.4	NA	Koo and Lee 2007
Rat	Oral	200	MEHP	Plasma	8.8	NA	Koo and Lee 2007
Rat	Oral	1,000	MEHP	Plasma	7.4	NA	Koo and Lee 2007
Rat	Oral	2,800	MEHP	Plasma	5.2	NA	Teirlynck and Belpaire 1985
Rat	Arterial	100	DEHP	Blood	15	1,290	Pollack et al. 1985a

Table 3-5. Blood, Serum, or Plasma Elimination Half-Lives ($t_{1/2}$) for DEHP and MEHP

Species	Route of administration[a]	Dose (mg/kg)	Measured chemical	Measured medium	Elimination $t_{1/2}$ (hour)	Clearance (mL/hour/kg)	Reference
Rat	Venous	50	[$^{14}CO_2$]	Blood	17[d]	NA	Tanaka et al. 1975
Rat	Venous	5	DEHP	Plasma	1.6[b]	571	Sjöberg et al. 1985b
Rat	Venous	10	DEHP	Plasma	3.2	NA	Chang-Liao et al. 2013
Rat	Venous	50	DEHP	Plasma	2.0[b]	514	Sjöberg et al. 1985b
Rat	Venous	500	DEHP	Plasma	3.8[b]	126	Sjöberg et al. 1985b
Pig	Oral	1,000	MEHP	Blood	6.3	NA	Ljungvall et al. 2004
After administration of MEHP							
Rat	Oral	400	MEHP	Plasma	5.5	NA	Teirlynck and Belpaire 1985
Rat	Oral	100	MEHP	Blood	2.8	NA	Pollack et al. 1985a
Rat	Venous	50	MEHP	Blood	3.2	690	Pollack et al. 1985a

[a]Single administration of compound.
[b]Effective $t_{1/2}$ calculated from mean residence time (MRT): ln[2] × MRT.
[c]MEHP elimination was quantified for two distinct phases: an initial fast elimination phase and a secondary slow elimination phase.
[d]Based on fitting blood-time data to a first-order exponential model.
[e][$^{14}CO_2$] represents the total for DEHP and its metabolites.

DEHP = di(2-ethylhexyl)phthalate; MEHP = mono(2-ethylhexyl)phthalate; NA = not available

After direct exposure to MEHP, reported blood and plasma elimination $t_{1/2}$ for MEHP range from 2.8 to 5.5 hours in rats (Pollack et al. 1985a; Teirlynck and Belpaire 1985).

Estimates of the urinary elimination $t_{1/2}$ for MEHP range from 2 to 8 hours in humans and from 6 to 18 hours in rats (Table 3-6) (Anderson et al. 2011; Kessler et al. 2012; Koch et al. 2004, 2005a; Koo and Lee 2007; Krais et al. 2018; Mittermeier et al. 2016). Koch et al. (2004, 2005a) estimated that the urinary $t_{1/2}$ in an adult human who received a single oral dose of 0.65 or 3.7 mg/kg DEHP was somewhat shorter for MEHP (2–5 hours) compared to its secondary metabolites (2–15 hours; see Table 3-6).

Table 3-6. Urinary Elimination Half-Lives ($t_{1/2}$) for DEHP, MEHP, and Metabolites

Species	Route of administration[a]	DEHP dose or concentration (mg/kg or µg/m^3)	Measured chemical	Elimination $t_{1/2}$ (hours)	Reference
Human	Inhalation	123	Sum of MEHP, MECPP, MEHHP, MEOHP, MEOPP	4.6	Krais et al. 2018
Human	Oral	0.00052 or 0.047	MEHP MECPP MEHHP MEOPP	4–8[b]	Anderson et al. 2011
Human	Oral	0.645	MEHP MEHHP MEOHP	4.6 6.6 6.2	Kessler et al. 2012
Human	Oral	3.7	MEHP MEHHP MEOHP	2–5 2–10 2–10	Koch et al. 2004
Human	Oral	0.65	MEHP MECPP MEOHP MEHPP	5 12–15 10 10	Koch et al. 2005a
Human	Oral	0.05 (MEHP)	MEHP MECPP MEOHP MEHPP	2.2–5.9 7.9–9.9 4.8–7.8 5.3–7.3	Mittermeier et al. 2016
Rat	Oral	200 1,000 5,000 200 1,000 5,000 40 200 1,000	MEHP MEHP MEHP DEHP DEHP DEHP [^{14}C][c] [^{14}C][c] [^{14}C][c]	18 6.0 6.4 ND 13 8.9 9.1 6.9 9.1	Koo and Lee 2007

[a]Single administration of compound.

Table 3-6. Urinary Elimination Half-Lives ($t_{1/2}$) for DEHP, MEHP, and Metabolites

Species	Route of administration[a]	DEHP dose or concentration (mg/kg or µg/m^3)	Measured chemical	Elimination $t_{1/2}$ (hours)	Reference

[a]Reported as a single range for all metabolites.
[b] ^{14}C represents the total for DEHP and its metabolites.

DEHP = di(2-ethylhexyl)phthalate; MECPP = mono-2-ethyl-5-carboxypentylphthalate; MEHP = mono(2-ethylhexyl) phthalate; MEHHP = mono-2-ethyl-5-hydroxyhexylphthalate; MEOHP = mono-2-ethyl-5-oxyhexylphthalate; ND = not detected

DEHP is measurable in blood and urine only after relatively higher doses of DEHP are administered orally (Kessler et al. 2004; Koo and Lee 2007; Pollack et al. 1985a; Sjöberg et al. 1986). Slower elimination $t_{1/2}$ values for DEHP relative to MEHP may reflect saturation of DEHP hydrolysis. Studies conducted in rats have demonstrated a dose-dependence of the kinetics of DEHP elimination. This was observed as a decrease in clearance and an increase in mean residence time and effective $t_{1/2}$ associated with increasing oral doses (4–2,000 mg/kg) (Koo and Lee 2007; Oishi 1989, 1990) or intravenous doses of DEHP (5–500 mg/kg) (Sjöberg et al. 1985b). Although the urinary elimination $t_{1/2}$ for MEHP remains relatively constant over dose ranges that begin to saturate DEHP elimination (Koo and Lee 2007), the dose-adjusted blood AUC for MEHP increases with increasing dose (Kessler et al. 2004). Contributing mechanisms for the higher plasma AUC may include saturation of pre-absorption hydrolysis of DEHP resulting in a larger and slower absorbed dose of DEHP, as well as possible saturation of systemic hydrolysis of DEHP. Both outcomes would contribute to a slowing of the time course for the elimination of MEHP from plasma.

Tanaka et al. (1975) reported data on the time course for ^{14}C in various tissues (male Wistar rats) following single intravenous (50 mg/kg) or oral (500 mg/kg) doses of [^{14}C]-DEHP (Tables 3-1 and 3-2). Based on these data, elimination $t_{1/2}$ values for blood and liver were approximately 17 and 8 hours, respectively, following the intravenous dose (predicted for this report from reported observations made 3–168 hours following the dose), and 11 and 10 hours following the oral dose (predicted for the observations made 3–24 hours following the dose; data for 168 hours were not reported). The $t_{1/2}$ for adipose following the oral dose was <10 hours; however, it could not be estimated following the intravenous dose because concentrations in adipose tended to remain the same or increase over time. Differences in the blood and tissue elimination rates of ^{14}C following intravenous and oral doses may reflect differences in the composition of the ^{14}C-labeled compounds in the systemic circulation. Following intravenous injection, a larger fraction of the systemic ^{14}C would have been comprised of [^{14}C]-DEHP, since pre-absorption hydrolysis would not have occurred. The more highly lipophilic DEHP

may have a longer residence time in adipose, which has a relatively low activity of DEHP hydrolase. See Section 3.1.2 for discussion of tissue distribution of DEHP hydrolase.

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

Several PBPK models of DEHP have been reported. These include a rat PBPK model that simulates the kinetics of orally administered DEHP and MEHP (Keys et al. 1999), a generic PBPK model and reported chemical parameter values for DEHP in rats (along with styrene, trichloroethene, and dibutylphthalate) (Cahill et al. 2003), an empirical model for predicting serum concentrations and urinary excretion of DEHP metabolites in humans (Lorber et al. 2010), a simplified humanized mouse model (Adachi et al. 2015), a human PBPK model that simulates the kinetics of orally administered DEHP (Sharma et al. 2018), and a human PBPK model that simulates the transfer of MEHP from the maternal system to the fetus (Martinez et al. 2018).

Keys et al. (1999)

Keys et al. (1999) developed a rat PBPK model that simulates the kinetics of orally administered DEHP and its metabolite, MEHP. Tissue compartments represented in the model include blood, fat, liver, small intestine, testes, slowly perfused tissues, and rapidly perfused tissues. The model simulates absorption of DEHP and MEHP in the small intestine as first-order transfer to liver. DEHP that is not absorbed is eliminated from the small intestine by a first-order loss parameter that represents fecal excretion. Hydrolysis of DEHP to MEHP in the small intestine is assumed to be capacity-limited and elimination of absorbed DEHP is assumed to be entirely by metabolism in liver and blood. Other viable elimination mechanisms for DEHP, including urinary excretion and biliary secretion, are not explicitly represented in the model, although they would have been at least partially represented in the metabolism parameters,

since these were optimized against blood DEHP elimination kinetics. Elimination of absorbed MEHP is assumed to be entirely by metabolism in the liver. As with DEHP, other elimination mechanisms for MEHP, including urinary excretion, are not simulated and would have been at least partially represented with the metabolism parameters for MEHP. Metabolites of MEHP are not simulated in the model.

Keys et al. (1999) explored three approaches to modeling the blood-tissue exchange of DEHP and MEHP: (1) flow-limited (with or without enterohepatic circulation); (2) diffusion-limited; and (3) intracellular pH trapping. Model performance was evaluated against observations of blood and tissue (liver, testes) MEHP concentrations in rats following single intravascular doses of DEHP or MEHP or repeated oral doses of DEHP (Oishi 1989, 1990; Pollack et al. 1985a; Sjöberg et al. 1985a; Teirlynck and Belpaire 1985). Simulation code was developed for Advanced Continuous Simulation Language (ACSLTOX, Pharsight) and parameter values were estimated using ACSLopt.

Keys et al. (1999) compared the performance of the various models using a log-likelihood ratio test with the flow-limited model as the reference. Significant improvement in the log-likelihood ratio was achieved for each alternative to the flow-limited model. The pH-trapping model was statistically better than all models and was selected for further evaluation. The model that assumed pH trapping without diffusion limitation consistently underpredicted observed blood concentration profiles. The diffusion-limited and enterohepatic flow-limited models gave comparable log-likelihood values. The enterohepatic circulation model was explored because delayed peaks in blood MEHP concentrations were evident in observations made in rats that received oral doses of DEHP (Kessler et al. 2004; Ljungvall et al. 2004; Pollack et al. 1985a; Sjöberg et al. 1985b). One contributor to a delayed peak in blood MEHP concentration could be the absorption of MEHP secreted in bile into the small intestine. Biliary secretion of MEHP has also been observed in rats following oral administration of DEHP (Daniel and Bratt 1974). Although the enterohepatic circulation model did produce a series of delayed peaks in blood MEHP concentration, the simulation did not offer an improved fit to the observed blood MEHP profile compared to the pH-trapping model.

Cahill et al. (2003)

Cahill et al. (2003) proposed a generic PBPK model and reported chemical parameter values for DEHP (along with styrene, trichloroethene, and dibutylphthalate). Parameter values were not optimized. Predictions from DEHP model are reported; however, evaluations of the model are limited to comparisons of predicted and observed mass balance (e.g., percentage of dose retained in body and

excreted in urine and feces) based on single-dose studies conducted in cynomolgus monkeys (Astill 1989) and rats (Daniel and Bratt 1974; Lake et al. 1984; Tanaka et al. 1978).

Lorber et al. (2010)

Lorber et al. (2010) reported a single-compartment model for simulating serum concentrations and urinary excretion of DEHP and metabolites in humans. The Lorber et al. (2010) model is not a PBPK model; however, it includes metabolism rates that could be useful for the development of PBPK models of MEHP metabolism. The model consists of two compartments, serum and urine, and one physiological parameter, volume of distribution in the serum compartment. Chemical parameters include first-order rate constants for each metabolic conversion of DEHP and MEHP, and deposition fractions of each metabolite representing the fraction of chemical mass transferred to bladder urine. Rates of change of the amount of chemical in the serum compartment are the sum of the products of the metabolism rates and deposition fractions.

Values for rate constants and deposition fractions were "optimized" against measurements made in a single adult subject who ingested 48.5 mg (0.65 mg/kg) DEHP-D_4 (Koch et al. 2005a), using a "trial and error" approach and not statistical goodness-of-fit evaluations. The model was evaluated against observations of DEHP metabolites excreted in urine of human platelet donors who received intravascular doses of DEHP from disposable PVC medical devices used in the donation process (Koch et al. 2005b). Dose reconstruction exercises were performed using this model and urinary biomarker data for DEHP metabolites collected from individuals in the general population (Lorber and Calafat 2012).

Adachi et al. (2015)

Adachi et al. (2015) developed a three-compartment model for simulating MEHP and its metabolite, MEHP-O-glucuronide (MEHP-O-G), in chimeric TK-NOG mice with humanized liver. The TK-NOG mouse strain expresses an inducible herpes simplex type 1 thymidine kinase, which destroys native hepatocytes. Immunosuppression of the mice allows human hepatocyte xenografts to establish liver function, with expression of human hepatocyte transporters, cytochrome P450, and UDP-glucanosyl-transferases (Hasegawa et al. 2011). Mice with humanized liver exhibited kinetics of plasma and urinary MEHP and MEHP-O-G following an oral dose of DEHP that were distinct from those of control mice: (1) faster clearance of MEHP and MEHP-O-G; (2) larger fraction of dose excreted in urine; and (3) larger fraction of dose converted to MEHP-O-G (Adachi et al. 2015). Control mice also exhibited biphasic

elimination from plasma with a delayed peak in plasma MEHP and MEHP-O-G concentrations, indicative of hepatobiliary recirculation that was not evident in mice with humanized livers.

The Adachi et al. (2015) model consists of two sub-models, one for MEHP and one for MEHP-O-G, which are linked by the conversion of MEHP to MEHP-O-G in the liver. An oral dose of DEHP is delivered to the liver compartment from the gastrointestinal tract (first-order k_a, $hour^{-1}$) where it is completely metabolized to MEHP and further metabolized to MEHP-O-G (first-order Cl_{int}, L/hour). Conversion of DEHP to MEHP is not simulated and, therefore, is treated as being essentially instantaneous. The central compartment represents blood, which is in equilibrium with plasma (R_b, blood-plasma concentration ratio). Transfers of MEHP and MEHP-O-G between the liver and central compartment are flow-limited (Q_h, L/hour; $K_{p,h}$, liver-plasma concentration ratio). MEHP and MEHP-O-G are eliminated from the central compartment by excretion into urine (first-order, Cl_r, L/hour). The liver compartment also includes an unspecified elimination pathway for MEHP-O-G (first order, Cl_{int}).

Adachi et al. (2015) estimated initial values for liver-plasma ($K_{p,h}$) and blood-plasma (R_b) concentration ratios and plasma binding ($f_{u,p}$) in mice from physical-chemical properties (Emoto et al. 2009; Poulin and Theil 2002). All other chemical parameter values for mice were estimated by optimization against data from oral dosing of mice with DEHP (Adachi et al. 2015) after initial values were assigned from the literature on studies of other chemicals in mice with humanized liver (Suemizu et al. 2014; Tsukada et al. 2013; Yamashita et al. 2014). In creating the human model, values for liver-plasma and blood-plasma concentration ratios were assumed to be the same in mice and humans. Intrinsic hepatic clearances were estimated for humans based on *in vivo-in vitro* ratios measured in mice (Adachi et al. 2015), with subsequent optimization against excretion data in humans (Kurata et al. 2012a).

Mouse model predictions were compared to observed kinetics of elimination of MEHP and MEHP-O-G from plasma following an oral dose of 250 mg/kg DEHP. Predictions were not significantly different from observations (chi-square, $p<0.001$). Human model predictions were compared to observed kinetics of MEHP and MHEP-O-G in urine, following an oral dose of 0.04 mg/kg DEHP. Predictions appeared to be close to observations (goodness of fit was not reported).

Applications for Dosimetry Extrapolation and Risk Assessment. The most fully advanced PBPK models for DEHP are those reported by Keys et al. (1999); however, these models have several important limitations for use in dosimetry predictions. The models simulate DEHP and MEHP kinetics in rats. An

analogous human model has not been proposed, although the Keys et al. (1999) model could be scaled to the human and optimized against observations in humans (Koch et al. 2005a). This precludes the use of the model, as currently developed, for interspecies extrapolation of DEHP dosimetry. All elimination of MEHP is attributed to liver metabolism; this precludes the use of extensive data on urinary excretion for evaluating model performance and would preclude the use of the model for translating urinary excretion data into predictions of DEHP intake (i.e., dose reconstruction). Other reported models are not useful in their current form for interspecies dosimetry predictions. The generic Cahill et al. (2003) model with metabolism parameters for DEHP is a rat model that has not been fully optimized or evaluated for performance. The largely empirical model proposed by Lorber et al. (2010) may be useful for predicting internal dosimetry of DEHP metabolites in humans; however, its structure will not support scaling to other animal species.

Adachi et al. (2015) used the human model to predict DEHP intakes that corresponded to observed urinary levels of MEHP in human populations (reverse dosimetry). Confidence in reverse dosimetry could be improved with more extensive evaluations of model predictions of dose-excretion relationships for MEHP in humans. Data used to evaluate predictions were from a single study of 20 subjects who received a single dose of DEHP (0.04 mg/kg). Another potential application of the model is for internal dose-response analysis using plasma MEHP as the dosimeter. The model provides predictions of plasma MEHP concentrations; however, model predictions of plasma concentrations in humans have not been evaluated against observations in humans.

Sharma et al. (2018)

Sharma et al. (2018) developed a human PBPK model that simulates the kinetics of orally administered DEHP. Tissue compartments represented in the model include blood, fat, liver, gut (absorptive regions), gonads, and a lumped compartment representing the rest of the body. The model simulates absorption of DEHP from the gut, distribution to tissues and elimination by metabolism, and urinary excretion of metabolites. Metabolic pathways simulated include formation of MEHP from DEHP and conversion of MEHP to MEHHP, MEHOP, MECPP, and phthalic acid. All metabolism pathways are assigned to the gut and liver. The model simulates the tissue distribution and urinary excretion of DEHP and MEHP, and the distribution to blood and urinary excretion of the metabolites MEHHP, MEOHP, and MECPP. The conversion of MEHP to phthalic acid is simulated as an elimination pathway; the distribution and excretion of phthalic acid is not simulated.

Absorption of DEHP from the gut is flow-limited and governed by a gut/plasma partition coefficient and blood (plasma) flow rate (L/hour). Rates of absorption of metabolites formed in the gut are governed by first-order rate coefficients (hour-1). Tissue distribution of DEHP and MEHP are assumed to be flow-limited, with rates governed by tissue/plasma partition coefficients and tissue blood flow rates (L/hour). Transfers of MEHHP and MEOHP to blood are assumed to be first-order (hour-1). All metabolic pathways are represented as saturable reactions acting on the unbound fraction in tissue, with rates governed by a K_m (µ/L) and V_{max} (µg/minute/mg microsomal protein). *In vivo* rates of metabolism are scaled to the mass of microsomal protein in each tissue. Urinary excretion of metabolites is assumed to be first-order (hour-1). Other viable elimination mechanisms for DEHP, including biliary secretion, are not explicitly represented in the model, although they would have been at least partially represented in the metabolism parameters, since these were optimized against plasma DEHP elimination kinetics.

Chemical parameters were assigned log-normal distributions representing uncertainty (see Table 1 of Sharma et al. 2018). The distributions were used in a Monte Carlo analysis to propagate parameter uncertainty into model outputs (e.g., plasma concentrations and amounts excreted in urine of parent compound and metabolites).

The model was optimized against observations of plasma and urine levels of DEHP and metabolites following a single oral dose of 48.5 mg DEHP (Koch et al. 2004, 2005a). Sharma et al. (2018) reported that central estimates for the first-order transfer coefficients of MEHHP and MEOHP to blood were optimized to observations. However, values of all other parameters estimated from other studies appear to have been optimized by adjusting their standard deviations to achieve 2.5 –97.5th percentile ranges of predictions that encompassed observations. The predicted 2.5th–97.5th percentile ranges encompassed the observed time course for plasma concentrations of MEHP, MEHHP, MECPP, and MEOHP. This indicates that the optimization of the uncertainty distributions was successful.

The model was evaluated against observation of urinary metabolite profiles following a single oral dose of 0.31 or 2.8 mg DEHP (Anderson et al. 2001). The predicted 2.5th–97.5th percentile ranges for the fraction of dose excreted in urine encompassed the observations for urinary MEHP, MEHHP, MECPP, MEOHP, and the sum of metabolites (Sharma et al. 2018).

Martinez et al. (2020) applied the Sharma et al. (2018) model to predicting cumulative urinary in a cohort of pregnant women. DEHP intakes from dermal application and ingestion of DEHP-containing products, inhalation, and diet were estimated from surveys of the cohort (Martinez et al. 2017, 2018). The

estimated total DEHP intakes were used as inputs to the Sharma et al. (2018) model to predict cumulative urinary excretion of MEHP, which were comparted to observations from biomonitoring (spot urine samples). The PBPK model underpredicted the median observed excretion of MEHP and predicted a narrower distribution of individual excretion (see Estimated exposure and Biomonitoring data in Figure 3 of Martinez et al. 2020). Closer agreement with biomonitoring data was achieved when dose inputs to the PBPK model were reconstructed for each subject from urinary excretion fractions (FUE, fraction of dose excreted in urine) previously estimated for each MEHP metabolite (Anderson et al. 2011). Predicted mean urinary excretion rates from the reconstructed doses were not different from observed (see Reconstructed exposure and Biomonitoring data in Figure 3 of Martinez et al. 2020). However, this comparison is not surprising given that the model was previously shown to predict the urinary excretion fractions observed in the Anderson et al. (2011) study (Sharma et al. 2018).

Martinez et al. (2018)

Martinez et al. (2018) extended the Sharma et al. (2018) model to simulate transfers from the maternal system to the fetus. The model includes compartments for placenta and fetus, and several additional maternal compartments not in the Sharma et al. (2018) model, including brain, fat, skin, and stomach; and placenta, fetus, and amniotic fluid. The model structure and parameter values are described in Annex-I of Martinez et al. (2017). However, the Annex provides only a partial description of the model; it does not provide a complete description of how the fate of metabolites, other than MEHP, are represented in the model. Transfers between plasma and tissues are assumed to be flow-limited and governed by tissue plasma flow rates and tissue/plasma partition coefficients (only those for DEHP and MEHP are reported in the Annex).

The fetus is simulated with compartments representing brain, liver, and rest of body. Transfer of MEHP to the fetus occurs from the placenta compartment, with the transfer assumed to be flow-limited and governed by placental blood flow and bidirectional transfer fractions of the unbound concentration in fetal and maternal plasma. Within the fetus, distribution of MEHP to tissue compartments is flow-limited. Elimination pathways for MEHP in the fetus include metabolism (V_{max}, K_m) and transfers between fetal liver and amniotic fluid, governed by bidirectional plasma-amniotic fluid transfer fractions. Metabolites of MEHP are not simulated in fetal compartments. Fetal growth is represented as exponential (fetal volume) or polynomial (amniotic fluid) of gestational age. Volumes of fetal tissues are proportions of fetal volume.

The maternal fetal model was used to simulate maternal and fetal plasma MEHP following a single dose of DEHP at levels representing the 4th and 95th percentile for dietary, non-dietary, and total DEHP intake estimated in a population of pregnant women (Martinez et al. 2018). Predicted peak concentrations in fetal and maternal plasma were similar; however, peak concentration occurred sooner (approximately 1 hour after dosing) in fetal plasma compared to maternal plasma (approximately 5 hours). Observations were not reported to allow evaluation of these predictions from the maternal-fetal model.

3.1.6 Animal-to-Human Extrapolations

The toxicokinetics of DEHP in humans are generally similar to those that have been observed in monkeys, rats, mice, hamsters, and guinea pigs. As discussed in Section 3.1.1, oral absorption data indicate absorption of 11–70% in humans and 30–78% in laboratory animals. No reliable data are available regarding distribution in humans. Metabolic pathways are similar between species (Figure 3-1), although species differences in relative abundance of metabolites and glucuronide conjugates have been reported. Extensive oxidative metabolism of MEHP was demonstrated to occur in rats compared to humans, and metabolites were primarily unconjugated in rat urine, whereas conjugation with glucuronide was extensive in humans (Albro et al. 1982a); see Section 3.1.3 for additional details. Species differences in DEHP hydrolase activities have been reported, with much lower activities in human and marmoset liver tissue compared with rodent liver tissue (Ito et al. 2005, 2014). In both humans and laboratory animals, elimination is primarily via excretion in urine and feces (Daniel and Bratt 1974; Koch et al. 2004, 2005a; Kurata et al. 2012a, 2012b). Elimination half-lives for DEHP and MEHP did not differ widely between species (Table 3-5).

Some DEHP-induced effects in rats and mice are thought to be mediated through the peroxisome proliferator-activated receptor-alpha (PPARα) (e.g., liver effects) and it is generally agreed that humans and nonhuman primates are refractory, or at least less responsive than rodents, to PPARα-mediated effects (Corton et al. 2018; Klaunig et al. 2003; Maloney and Waxman 1999). However, many of the health effects associated with DEHP and its metabolites in rodents (e.g., reproductive effects) are believed to act through other mechanisms that are independent of PPARα activation, which may be also relevant for exposed human populations.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental

germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to DEHP are discussed in Section 5.7, Populations with Potentially High Exposures.

Age-Related Exposure and Pharmacokinetic Differences. Efforts to reduce and/or regulate the use of DEHP in cosmetics, food contact materials, and toys, have reduced all exposures to DEHP in the United States and Europe, including children's exposure (Johns et al. 2016). In 2008, the U.S. Consumer Product Safety Improvement Act restricted the amount of DEHP in children's toys and childcare products to ≤0.1% (Johns et al. 2016). Coupled with earlier actions by the European Union to prohibit the use of DEHP in other consumer products and public awareness of the issue, this action has led to the reformulation of many consumer products to limit or eliminate DEHP, sometimes substituting other phthalate esters (Johns et al. 2016). Thus, infant and toddler exposures have likely decreased, although biomonitoring data over time for these age groups are limited. However, mouthing behaviors of infants and toddlers may still lead to higher DEHP exposures than experienced by older children or adults.

No specific information was located regarding the comparative absorption of DEHP in children and adults. In rats, oral absorption of DEHP appears to be greater in immature animals compared with mature animals (Sjöberg et al. 1985a), but no age-related differences in oral absorption were seen in marmosets (Kurata et al. 2012b). Age-related differences in metabolism may also contribute to variations in susceptibility. The metabolism of DEHP to MEHP is mediated by lipases that are mainly in the gastrointestinal tract. Gastric lipase activity is high in infants to aid in the digestion of fats in milk, peaking in children at 28–33 weeks of age (FDA 2001; Lee et al. 1993). Consequently, young children might convert DEHP to MEHP more efficiently than older children or adults (FDA 2001). In addition, compared to adults, children generally have a reduced capacity to metabolize compounds via glucuronidation (FDA 2001). Since approximately 60% of an administered dose of DEHP is excreted as

the glucuronide conjugate in humans (Albro et al. 1982a, 1982b), a reduced glucuronidation capacity could result in delayed excretion of DEHP or its metabolites. The MEHP metabolite of DEHP also undergoes glucuronidation and has been shown to interfere with bilirubin conjugation (Sjöberg et al. 1991), possibly as a competitive inhibitor of glucuronidase (FDA 2001).

Age-Related Differences in Susceptibility. As detailed in Chapter 2, epidemiological and/or animal studies have suggested that exposure to DEHP may lead to numerous developmental effects, including preterm birth, fetotoxicity, teratogenicity, effects on the male reproductive system, early puberty, and altered development of the nervous, endocrine, hepatic, and renal systems. The developing male reproductive system appears to be a particularly sensitive target for DEHP.

Studies directly comparing the effects of DEHP exposure in humans or animals of different ages are few but confirm the greater susceptibility of younger organisms. For example, acute DEHP doses associated with lethality are lower in younger rats (Dostal et al. 1987; Tonk et al. 2012). Two oral doses of 2,000 mg/kg/day DEHP caused nearly 100% mortality in ≤21-day-old rats, but no mortality in ≥42-day-old rats (Dostal et al. 1987). In addition, five daily doses of 1,000 mg/kg DEHP resulted in 66–70% mortality in rats exposed on PNDs 6–10, 16–20, or 21–25, but not in those exposed at ages ≥PND 42. Similarly, several PND 10 pups died within 1 day receiving a dose of 1,000 mg/kg DEHP, while no mortality was seen in PND 50 animals receiving the same dose for 40 consecutive days (Tonk et al. 2012).

Studies in male rats of different ages demonstrate the increased susceptibility of younger (≤PND 35) rats to DEHP-induced effects on the male reproductive system (Murphy et al. 2014; Sjöberg et al. 1985b; Tonk et al. 2012). For example, Tonk et al. (2012) exposed male Wistar rats exposed to DEHP by gavage for 40 days, beginning at either PND 10 or 50. A broad range of doses from 1 to 1,000 mg/kg/day was administered to both groups. The juvenile rats exhibited significantly decreased androgen-dependent organ weights (testes, epididymides, and ventral prostate) at lower doses than adult rats, while effects on liver and kidney weights occurred at the same dose for both juveniles and adults. In addition, serum LH and FSH levels were markedly increased in juvenile rats, but not adult rats, while serum testosterone changes occurred at the same dose and magnitude of response at both ages (Tonk et al. 2012). Similar findings were reported by Sjöberg et al. (1985b), who observed testicular damage in rats exposed to DEHP at 1,000 mg/kg/day for 14 days beginning at PND 24, but not when exposure was begun at PND 40 or 60.

Age-dependent susceptibility to testicular effects was also seen in rats after exposure to the DEHP metabolite, MEHP (Murphy et al. 2014; Teirlynck et al. 1988). Murphy et al. (2014) compared effects of oral exposure to MEHP (1 g/kg) in mouse and rat testes after single exposures on PNDs 21, 28, 35, or 56. In rat testes, increased infiltration of immunoreactive interstitial cells (mediated by increased production of monocyte chemoattractant protein-1) and increased apoptosis were seen after dosing in juvenile rats, but not adult (PND 56) rats. Effects occurred earlier in younger (PND 21 and 28) juveniles (e.g., within 12 hours after dosing, compared with 48 hours) than in older (PND 35) juveniles (Murphy et al. 2014). Similarly, testicular damage was observed in rats given a single dose of 800 mg/kg MEHP on PND 25, but not when MEHP was administered on PND 44 or 71 (Teirlynck et al. 1988).

Age-dependent sensitivity to DEHP-induced effects on the hypothalamic-pituitary-adrenal (HPA) axis and steroidogenesis has also been demonstrated. When male rats were exposed to DEHP on 4 consecutive days beginning on PND 16, 36, or 56, significant increases in adrenocorticotropic hormone (ACTH) and cortisone were seen in the younger rats, but not in the rats exposed as adults (PND 56) (Supornsilchai et al. 2007). In addition, adrenocortical cells from rats exposed at PNDs 16 and 36 showed increased steroidogenesis compared with cells from rats exposed as adults, as shown by greater corticosterone production in response to stimulation by ACTH, dibutyryl cAMP, and 22R-hydroxy-cholesterol, and greater transportation of cholesterol into mitochondria (Supornsilchai et al. 2007).

In addition to increased susceptibility to male reproductive and adrenal effects, juvenile rats exhibit greater sensitivity to immune system perturbations induced by DEHP. In male Wistar rats exposed to DEHP by gavage from PND 10 to 50 or from PND 50 to 90, immune system endpoints were affected at a lower dose in juvenile rats than adults (Tonk et al. 2012). Effects seen in juvenile rats included decreases in white blood cells, neutrophils, lymphocytes, and monocytes, and increases in KLH-stimulated cytokine production. Adult rats exhibited some, but not all, of these effects at higher doses (Tonk et al. 2012).

Transgenerational Effects. There is no information regarding possible transgenerational effects of DEHP in humans. However, studies in animals showed transgenerational effects on gonad development in both male and female descendants, possibly resulting from epigenetic changes in the germ cells.

In male descendants of rats exposed to DEHP, effects included cryptorchidism, impaired fertility, and effects on testicular structure and function (Chen et al. 2015; Doyle et al. 2013; Quinnies et al. 2015). Chen et al. (2015) observed increased incidences of cryptorchidism, decreased AGD, and decreased testes and epididymides weights in both F1 and F2 (but not F3 or F4) generation Sprague-Dawley rats, after

DEHP exposure limited to the F0 generation dams (750 mg/kg/day from GD 7 to 19). Testes from both F1 and F2 rats in the DEHP-exposed line exhibited significantly increased expression of mRNA for three DNA methyltransferases compared with controls, while no treatment-related changes were seen in the F3 and F4 generations. It was suggested that DNA methylation changes might be responsible for the transgenerational effects on rat testes (Chen et al. 2015). Further evidence for transgenerational effects of DEHP exposure on testicular structure and function comes from a study in CD-1 mice (Doyle et al. 2013). F0 mice were exposed to 500 mg/kg/day DEHP by gavage from GD 7 to 14. The F1 mice were used in three experiments examining maternal (F1 females bred with untreated males), paternal (F1 males bred with untreated females), and double-cross (F1 males and females bred within exposure group) inheritance patterns. Male F2 and F3 offspring of paternal and double-cross groups from the DEHP exposure line exhibited significantly delayed pubertal onset; offspring of the maternal DEHP exposure inheritance line did not show a change in onset of puberty. In addition, F2, F3, and F4 offspring of all three exposure inheritance lines displayed increased numbers of abnormal seminiferous tubules and decreased epididymal sperm counts and sperm motility. The authors also conducted experiments in which germ cells from F3 offspring were transplanted into recipient testes; these experiments showed markedly reduced germ-cell recovery of spermatogenesis in the DEHP-exposed inheritance group compared with offspring of the control group. In addition, the testes of animals receiving germ cells from the exposure line exhibited morphology that resembled that of DEHP-exposed F1 offspring (i.e., tubules were disorganized, lacked layers of germ cells, and contained vacuoles and/or multinucleated cells), while testes of animals receiving germ cells from the control line exhibited normal morphology. Based on this observation, Doyle et al. (2013) postulated that the testicular phenotype has its origin in the F3 offspring stem cells.

Transgenerational effects of DEHP exposure on ovarian development were observed in mice (Zhang et al. 2015). When pregnant CD-1 mice (F0 generation) were given oral doses of DEHP at 0.04 mg/kg/day throughout gestation, effects on ovarian development were seen not only in the F1 offspring, but also in F2 generation females; the numbers of primordial follicles were significantly decreased, and numbers of secondary follicles increased, compared with control mice with ancestors that were not exposed to DEHP (Zhang et al. 2015). After observing that F1 females exhibited significantly increased methylation of the *Stra8* gene (stimulated by retinoic acid gene 8, *Stra8* is expressed in the embryonic mouse germ cells and is important to the initiation of meiosis), along with decreased levels of *Stra8* mRNA, the authors suggested that modification of DNA methylation patterns may play a role in the transgenerational effects of DEHP on ovarian development.

Genetic Polymorphisms Altering Susceptibility. Genetic polymorphisms that may increase susceptibility to the effects of DEHP have been examined in a few epidemiological studies, but most of these studies were cross-sectional in design, providing an inadequate basis with which to draw clear conclusions. Xie et al. (2015) reported that the association between MEHP levels in meconium and low birth weight or short birth length was enhanced in infants exhibiting the paraoxonase-2 148AG/GG (PON-2 A148AG/GG) genotype (PON-2 deficiency is associated with increased ROS levels). DEHP exposure (measured as urinary metabolites) was associated with greater decreases in lung function in elderly Koreans who exhibited certain polymorphisms in oxidative stress-related genes (CAT, MPO, and SOD2) (Park et al. 2013)

Park et al. (2014) investigated potential genotype-phthalate interactions between urinary levels of phthalate metabolites (including MEHP and MEOHP) and polymorphisms at major candidate genes for attention-deficit/hyperactivity disorder (ADHD) with regard to neuropsychological performance in 179 Korean children with ADHD. An increased in DEHP urinary metabolites was associated with poor attentional performance in children with the dopamine receptor D4 (DRD4) gene 4/4 variant, but not in children without the DRD4 4/4 genotype. This suggests that the DRD4 4/4 genotype may increase susceptibility to the effects of DEHP.

The potential for increased susceptibility to DEHP in individuals with loss-of-function filaggrin gene (FLG) variants has also been evaluated (filaggrin is an epidermal protein important to maintaining normal skin function, and its loss may enhance absorption of xenobiotics or allergens). No relationship between DEHP and atopic dermatitis was observed in individuals with or without FLG variants (Wang and Karmaus 2015). Additionally, internal body burden of DEHP (as measured by urinary metabolite levels) was not altered in persons with FLG variants (Joensen et al. 2014).

In a case-control study (Martinez-Nava et al. 2013), the associations between urinary DEHP metabolite levels and breast cancer were stronger in individuals with polymorphisms in *PPARγ* (shown previously to modify breast cancer risk) and PPARγ coactivator 1 beta *(PPARGC1B*, a co-activator of estrogen receptor α that amplifies ER signaling). However, since exposure was measured after the individuals developed breast cancer in this study, the findings were not considered to be useful for assessment of cancer hazard for DEHP, and thus, the potential roles of PPARγ and its coactivator remain unknown. In another case-control study of women with uterine conditions (endometriosis, adenomyosis, or leiomyoma), Huang et al. (2010) observed a significant association between MEHP in urine and odds of leiomyoma or

adenomyosis only in individuals with GSTM1 null-type polymorphisms and not in those with wild-type GSTM1.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to DEHP are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (http://www.cdc.gov/exposurereport/). If available, biomonitoring data for DEHP from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by DEHP are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

As discussed in Section 3.1, DEHP is rapidly and extensively hydrolyzed to MEHP within the gastrointestinal tract, and both DEHP and MEHP (formed in the gastrointestinal tract) are readily absorbed. Systemically absorbed DEHP may undergo hydrolysis to MEHP by tissue lipases found in many tissues; in addition, MEHP may be oxidized, yielding MEHHP, MEOHP, and MECPP. The oxidized metabolites of MEHP are primarily conjugated with glucuronic acid and excreted in the urine. Hydrolysis of absorbed DEHP to MEHP is sufficiently rapid that, regardless of the route of administration of DEHP, most of the phthalate eliminated from the body is in the form of MEHP and its metabolites. Elimination of MEHP and its oxidative metabolites occurs via urinary and biliary excretion.

It is generally agreed that the preferred biomarkers for exposure to DEHP are its urinary metabolites (Calafat et al. 2015; Johns et al. 2016). While modern analytical techniques permit the detection and quantification of DEHP and its metabolites in serum, amniotic fluid, meconium, breast milk, and semen, there are several advantages to using metabolites in urine over measurement of DEHP or its metabolites in other biological fluids. First, urine samples are the least invasive samples to obtain, improving participation in efforts to assess exposure. Second, urine samples are typically of larger volume than those of other biological fluids, facilitating detection of metabolites. Third, the concentration of DEHP metabolites in urine is higher than that of DEHP or its metabolites in other biological fluids, leading to fewer samples below the limit of detection. Fourth, while DEHP can be detected in these media, enzymes present in blood, milk, amniotic fluid, etc., but not in urine, are known to hydrolyze DEHP to its monoester during sample storage, leading to underestimates of DEHP levels. Further complicating the analysis of DEHP in biological fluids is the significant potential for contamination from materials used to store samples and/or in the laboratories where analyses are performed. The direct measurement of metabolites in urine reduces the potential for sample contamination by the parent diester and subsequent metabolism by enzymes found in blood, milk, and amniotic fluid, but not urine (Johns et al. 2015).

While urinary metabolites are considered the optimal biomarkers for DEHP exposure, these metrics are also subject to uncertainties that should be considered in assessing DEHP exposure (Johns et al. 2016). For example, urinary metabolites of DEHP vary over time, with concentrations increasing over the course of the day as well as between days. In studies assessing temporal variability, intraclass correlation coefficients (ICCs; reflecting the variance between individuals divided by the sum of the variances between and within individuals) for DEHP metabolites in urine have been relatively low (on the order of 0.1–0.3; Johns et al. 2016) over short time periods (up to 1 month) and lower over longer time periods (1–

3 years). Evaluations of ICCs for individual or summed DEHP metabolites during pregnancy have reported values from 0.08 (Braun et al. 2012) to 0.22 (Peck et al. 2010). The within-woman ICC values for individual metabolites measured during the three trimesters of pregnancy ranged from 0.21 to 0.44, suggesting low to moderate variability (Li et al. 2019a). Despite the temporal variability, single urine samples have been shown to provide reasonable prediction of exposure category (e.g., whether a given person's exposure is above or below the median or quartile of exposure level; Johns et al. 2016). Due to the potential for significant temporal variability, repeated urine samples are recommended to examine long-term exposure.

One study has shown that the intra-individual variability over a week in MEHHP concentrations from repeated spot urine samples is comparable to the intra-individual variability obtained from repeated first morning or 24-hour urine samples, indicating that spot urine samples remain useful for exposure assessment where 24-hour void samples are not feasible (Johns et al. 2016). However, a limitation of spot urine samples as biomarkers of exposure is the issue of urine dilution: the concentration of a given metabolite in urine will depend on the volume of urine, which in turn varies by time of day, water intake, physical activity, and sweating, as well as other factors unrelated to exposure (Johns et al. 2016). Efforts to address this limitation include adjustment for dilution using creatinine levels and specific gravity. Specific gravity adjustment is preferred over creatinine adjustment, because creatinine levels vary by an individual's activity level, time of day, age, gender, muscle mass, and medical conditions, while specific gravity is a more stable measure of dilution (Johns et al. 2016).

DEHP is rapidly metabolized to MEHP, but typically <10% of an oral dose of DEHP is eliminated in the urine as MEHP; most of the dose is excreted as oxidative metabolites including MEHHP, MEOHP, and MECPP (Johns et al. 2016). Thus, the concentration of the monoester MEHP alone is not considered an adequate measure of exposure (Johns et al. 2016). While phthalic acid can be quantified in urine, this is a nonspecific biomarker of DEHP exposure, since other phthalate esters such as butyl benzyl phthalate, dibutyl phthalate, and diethyl phthalate will also result in measurable phthalic acid in the urine. Recently, efforts to identify a single metric of DEHP exposure have focused on either the sum of the primary DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP), the percent of the sum attributable to MEHP (MEHP%), or the ratio of MECPP to MEHHP as valuable metrics. As reported by Johns et al. (2016), MEHP% may be an indicator of an individual's capacity to further metabolize the monoester, which is believed to be more bioactive than its oxidative metabolites. The ratio of MECPP to MEHHP is thought to provide a measure of the duration of time since exposure to DEHP, based on the half-lives of each of these metabolites (Johns et al. 2016).

Despite the limitations, urinary concentrations of DEHP metabolites are currently considered the optimal biomarkers for exposure. Based on studies of the sensitivity and specificity of a single sample to correctly classify categories (e.g., highest tertile versus lowest) of exposure. Johns et al. (2016) conducted sensitivity and specificity studies to evaluate the ability of a single urine sample to correctly classify categories (e.g., highest tertile versus lowest) of exposure. Based on the results of these studies, Johns et al. (2016) concluded that a single urine sample provides a reasonable means of categorizing an individual's exposure over several months or possibly up to 1 or 2 years. Little information is available on the identification of biomarkers that more accurately reflect long-term or cumulative exposure to DEHP. Camann et al. (2013) postulated that DEHP metabolite levels in deciduous teeth might serve as a marker for early childhood exposure. MEHP was detected in the molars of 29% of 21 children, and levels were higher in older than younger children, consistent with accumulation with longer exposure. However, the use of DEHP metabolites in teeth as a biomarker of exposure has not been validated.

3.3.2 Biomarkers of Effect

No specific biomarkers of the effects of exposure to DEHP were identified in the available literature.

3.4 INTERACTIONS WITH OTHER CHEMICALS

There are no studies in humans examining interactions between DEHP and other chemicals; however, most available human studies examined members of the general population with potential exposures to other phthalates as well as other ubiquitous chemicals.

Interactions Potentially Influencing Male Reproductive Toxicity. The majority of available interaction studies focused on potential interactions between DEHP and other chemicals with respect to adverse effects on the adult or developing male reproductive system. A number of studies focus specifically on the potential interactions between DEHP and other phthalate esters. Due to the similarities between the different phthalates, NAS recommends a cumulative risk assessment approach to determining the risks posed by phthalates (NAS 2008).

Available evidence from two well-designed oral interaction studies in rats indicates that phthalate esters act in a dose-additive manner with respect to developmental male reproductive toxicity (Hannas et al. 2011; Howdeshell et al. 2008). Both studies were adequately designed to evaluate interactions, including dose-response analyses for individual chemicals as well as the tested mixture. Howdeshell et al. (2008)

evaluated the dose-response effects of benzobutyl phthalate (BBP), di(n)butyl phthalate (DBP), DEHP, diisobutyl phthalate (DIBP), and dipentyl phthalate (DPP) on *ex vivo* fetal testicular testosterone (FTT) production in Sprague-Dawley rats following maternal exposure to individual phthalates at various doses from GD 8 to 18. FTT data from these experiments were used to build a dose-addition model, which accurately predicted FTT data following maternal exposure to various doses of the five-phthalate mixture (a set 3:3:3:3:1 mixture ratio for BBP:DBP:DEHP:DIBP:DPP was used for equipotency). Using a similar experimental design, Hannas et al. (2011) also observed that dose-additivity model predictions provided the best fit to FTT data from Sprague-Dawley rats following maternal exposure to a mixture of nine phthalates, including DEHP, DIBP, DBP, BBP, DPP, diisoheptyl phthalate, dicyclohexyl phthalate, diheptyl phthalate, and dihexyl phthalate, from GD 14 to 18.

Findings from other studies also suggest dose additivity between DEHP and DBP for additional reproductive development effects in male rats (malformations, androgen-dependent organ weights, gene expression) (Howdeshell et al. 2007; Martino-Andrade et al. 2009); however, study designs were inadequate to characterize potential interactions (lack of dose-response data for individual phthalates and/or mixture). Taken together, these findings support the hypothesis that phthalates share a common mechanism of action.

With regard to shared mechanisms, several *in vitro* and *in silico* studies have measured phthalate binding to various receptors (androgen, progesterone, glucocorticoid, sex hormone-binding globulin [SHBG], CAR, PXR, PPAR), binding to enzymes in the glucocorticoid biosynthesis pathway, and toxicogenetic signatures in an effort to predict how phthalates may interact with one another and to better inform cumulative risk assessments (Ahmad et al. 2017; Laurenzana et al. 2016; Sarath Josh et al. 2016; Sheikh et al. 2016; Singh and Li 2011). However, none of these studies speak to the potential nature of the interaction between phthalates.

Studies have also been conducted to evaluate potential interactions between DEHP and non-phthalate endocrine disruptors. Christiansen et al. (2009) evaluated several male reproductive endpoints in Wistar rats following maternal exposure from GD 7 to PND 16 to known androgen disruptors with different proposed mechanisms of action, including DEHP, vinclozolin (androgen receptor agonist), prochloraz (androgen receptor antagonist, inhibition of progesterone conversion to testosterone), and finasteride (androgen receptor agonist). Dose-response studies were conducted for individual chemicals as well as the mixture, and evaluated endpoints included AGD, nipple retention, external malformations, and sex organ weights. The mixture ratio of vinclozolin:finasteride:DEHP:prochloraz was set at

500:1:300:500 for equipotency of chemicals based on NOAELs determined in individual compound studies. Based on statistical analysis, both dose-addition and independent action models underpredicted the incidence of dysgenesis of the external genitalia in male offspring at PND 16 and 47, suggesting a synergistic or greater-than-additive effect (Christiansen et al. 2009). However, dose-additivity models accurately predicted the data for other endpoints (AGD, nipple retention, organ weights). Similarly, Fiandanese et al. (2016) reported a synergistic (or greater-than-additive) effect between DEHP and a mixture of polychlorinated biphenyls (PCBs) in the development of gross and histopathological changes in the testes of male offspring of mouse dams exposed to the mixture during gestation and lactation, and they reported "non-interaction" for sperm parameters or testosterone production. However, the study design was not adequate to properly characterize the nature of chemical interactions (single dose only for individual chemicals and mixture). In a cohort of male partners of infertile couples, Hauser et al. (2005) did not find a significant relative excess risk due to interaction (RERI) for below-normal sperm parameters between urinary MEHP levels and various serum PCB levels.

Jarfelt et al. (2005) evaluated potential interactions between DEHP and the proposed substitute chemical, di(2-ethylhexyl)adipate (DEHA), on the developing male reproductive system. Pregnant Wistar rats were exposed to DEHP alone at 300 or 750 mg/kg/day or DEHP (750 mg/kg/day) + DEHA (400 mg/kg/day) from GD 7 to PND 17, and male offspring were examined for AGD, nipple retention, sex organ weights, and testicular histology. The study authors concluded that there was no evidence for interaction between DEHP and DEHA because male reproductive effects were similar in the 750 mg/kg/day DEHP-only group and the DEHP+DEHA group; however, the study design is inadequate to fully characterize potential interactions.

A series of studies evaluated the influence of the phytoestrogen genistein on DEHP-induced male reproductive toxicity (Jones et al. 2014, 2015, 2016; Zhang et al. 2013, 2014). Results from these studies have been conflicting, and the designs of most studies were inadequate to establish the nature of the potential interactions.

Zhang et al. (2014) examined AGD, sex organ weight, testicular histology, and oxidative stress in adult rats exposed to genistein at 50 mg/kg/day, DEHP at 50, 150, or 450 mg/kg/day, or genestein+DEHP (at each DEHP dose level) from PND 22 to 32 (prepubertal exposure). Genistein alone did not affect any measured parameter; however, it significantly decreased several adverse effects observed with DEHP exposure, including sex organ weight, testicular oxidative stress, and testicular histopathological changes.

The study authors proposed that enhancement of testicular antioxidative enzyme activities by genistein protected against DEHP-induced testicular toxicity.

Jones et al. (2015) also observed partial alleviation of DEHP-induced alterations in testicular gene expression in neonatal male offspring of pregnant rats exposed to 10 mg/kg genistein plus 10 mg/kg/day DEHP from GD 14 through parturition, compared with 10 mg/kg/day DEHP alone. However, when adult offspring were evaluated following the same exposure scenario, long-term alterations in the male reproductive system (increased testicular weights and altered testicular gene expression suggestive of altered testicular function and spermatogenesis) were observed only in the DEHP+genistein group (Jones et al. 2014). Similar effects on steroid production and lipid homeostasis were observed with combined exposure to mouse tumor Leydig cells *in vitro* (Jones et al. 2016).

Zhang et al. (2013) also reported potential enhancement of DEHP-induced male reproductive effects with coexposure to genistein. While exposure-related changes in offspring AGD, testicular histology, testosterone levels, or testicular gene expression were not observed following maternal exposure to 250 mg DEHP/kg/day, 50 mg genistein/kg/day, or 400 mg genistein/kg/day alone from GD 3 to PND 21 in Sprague-Dawley rats, dose-related changes were observed in these endpoints following exposure to 250 mg DEHP/kg/day plus 50 or 400 mg genistein/kg/day. The study authors concluded that genistein and DEHP acted in a cumulative manner.

The potential effect of acetone on the testicular toxicity of DEHP was evaluated in in male Wistar rats in a 4-week oral study (Dalgaard et al. 2000). Rats were exposed to 0, 1,000, 5,000, or 10,000 mg/kg/day for 4 weeks or 0, 125, 250, 500, or 1,000 mg/kg/day DEHP for 9 weeks with or without 0.5% acetone. Male reproductive endpoints evaluated in the study included male fertility (4-week study only) and sex organ weight and histology. A significant, dose-related decrease in male fertility was observed with DEHP exposure; this effect was not significantly altered by co-exposure to acetone. No significant changes were observed in male reproductive organ weight or histology in the 9-week study following DEHP or DEHP+acetone exposure. In the 4-week study, decreased testes weight and increased incidence of testicular histopathological lesions were observed at ≥5,000 mg DEHP/kg/day, both with and without acetone. Analysis showed no significant interaction between DEHP and acetone with respect to organ weight; however, degeneration of the seminiferous tubules was “apparently” increased by acetone. The study authors did not present statistical analysis of potential interaction between DEHP and acetone with regard to testicular degeneration. Overall, the study concluded that there was no significant interaction between DEHP and acetone with respect to male reproductive toxicity.

In an *in vitro* study with a full-factorial design (all possible combinations tested at multiple concentrations), no clear evidence of synergism with respect to glucocorticoid-like activity in MDA-kb2 cells was observed using binary, trinary, or quaternary mixtures containing DEHP, propylparaben, butylparaben, and tetramethrin; all individual compounds showed glucocorticoid-like activity (Klopcic et al. 2015).

Interactions Potentially Influencing Developmental Toxicity. In the dose-response study by Howdeshell et al. (2008) described above, phthalates (BBP, DBP, DEHP, DIBP, and DPP) acted in a dose-additive manner for fetal toxicity in Sprague-Dawley rats following maternal exposure from GD 8 to 18. Decreased litter size and postnatal survival were also observed in rats exposed to DEHP+DEHA, compared with DEHP-only groups, in the study by Jarfelt et al. (2005) described above. However, since there was no DEHA-only group, no conclusions regarding interactions can be made.

Interactions between DEHP, trichloroethylene, and heptachlor on developmental toxicity have been investigated (Narotsky and Kavlock 1995). The compounds were administered to pregnant rats from GD 6 to 15 via gavage, singly and in combination, using five dose levels of each in a 5x5x5 factorial design. The dose levels were 0, 24.7, 78, 247, and 780 mg/kg/day for DEHP; 0, 10.1, 32, 101, and 320 mg/kg/day for trichloroethylene; and 0, 0.25, 0.8, 2.5, and 8 mg/kg/day for heptachlor. Endpoints that were analyzed for possible interactions included maternal death, maternal body weight gain on GDs 6–8 and 6–20, full-litter resorption, prenatal loss, postnatal loss, pup body weight on PNDs 1 and 6, and pups/litter with eye defects. Statistical analysis of the three maternal and six developmental endpoints yielded several significant two-way interactions. DEHP and heptachlor showed synergism for maternal death on GDs 6–8 and antagonism for maternal weight gain on GDs 6–8, full-litter resorption, and pup weight on PNDs 1 and 6. DEHP and trichloroethylene were synergistic for maternal weight gain on GDs 6–8, prenatal loss, and pup weight on PND 6. No significant three-way interactions were observed.

A combination of 150 mg/kg caffeine administered by injection to pregnant rats in conjunction with a single dose of 9,756 mg/kg DEHP on GD 12 caused a 5-fold increase in the number of dead and resorbed fetuses and nearly a 4-fold increase in the malformed survivors, as compared to the effects of DEHP alone (Ritter et al. 1987). The mean fetal weight was also depressed. The addition of the caffeine to the treatment using equimolar quantities of 2-ethylhexanol and 2-ethylhexanoic acid at doses half of the

molar quantity used for DEHP resulted in 2- to 30-fold increases in the dead and malformed fetuses and malformed survivors, but only minor decreases in the fetal weights.

Interactions Potentially Influencing Neurotoxicity. Interactions between DEHP, trichloroethylene-, and heptachlor-induced neurotoxicity were investigated in the study by Moser et al. (2003) described earlier. Neurobehavioral endpoints that were analyzed for possible interactions of the three chemicals included automated motor activity analysis in a figure-eight maze and an abbreviated FOB (general appearance, open-field observation, sensorimotor responses to click stimulus, pinch, and penlight stimulation, and grip strength); potential interactions were analyzed using a statistical response-surface analysis. No exposure-related changes in neurobehavior were observed with DEHP exposure alone, while various alterations were associated with trichloroethylene or heptachlor exposure. In two-way analyses, no significant interaction was observed between DEHP and trichloroethylene in any of the measures or DEHP and heptachlor for most measures. The one exception was evidence for a greater-than-additive effect between DEHP and heptachlor for tremors. In the three-way analysis, evidence for an antagonistic interaction was observed for the tail-pinch response; no other significant interactions were observed in neurobehavioral endpoints. Lethality was also assessed in this study, with DEHP exerting a less-than-additive effect on heptachlor-induced lethality. In the three-way analysis, there was evidence for a greater-than-additive effect on lethality.

In the 4-week study by Dalgaard et al. (2000) evaluating potential interactions between DEHP and acetone described in the male reproductive section above, a FOB was conducted. No exposure-related effects were observed in the 9-week study. In the 4-week study, acetone exposure was associated with significant decreases in hind limb grip strength and DEHP exposure was associated with significant decreases in forelimb grip strength; however, there was no significant interaction between the two chemicals.

The potential interactions between DEHP, bisphenol A (BPA), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on neurodevelopment were evaluated in ICR mouse offspring following maternal exposure to 1 mg DEHP/kg/day, 5 mg BPA/kg/day, 8 ng TCDD/kg/day, or their mixture during gestation (GDs 8–17 for BPA and DEHP, GD 8 only for TCDD) and lactation (GDs 3–7 BPA or DEHP, single exposures, or GDs 3–5 BPA and DEHP, mixture). TCDD exposure was only once due to its extended biological half-life. Endpoints examined were limited to markers of dopamine and neuronal activation in the midbrain. While significant alterations were observed with individual chemical exposures, none were observed

following exposure to the mixture. The study authors suggested that this was presumably due to antagonistic effects; however, the study design was not adequate to rigorously assess interaction.

Interactions Potentially Influencing Liver Toxicity. Data are available suggesting that DEHP might act as an antagonist for the hepatic damage caused by TCDD. DEHP was combined with TCDD to determine if the hypolipidemic effects of DEHP could counteract the hyperlipidemic effects of the TCDD (Tomaszewski et al. 1988). Pretreatment with DEHP mitigated many of the toxic effects of TCDD. There was a 50% decrease in TCDD-related mortality when the rats received DEHP pretreatment. DEHP administered after TCDD administration had considerably less of an effect on TCDD toxicity, but it did alleviate the TCDD toxic effects to a slight extent. The authors postulated that the antagonist properties of DEHP could have resulted from either or both of two mechanisms: (1) reduction in TCDD-induced hyperlipidemia by DEHP stimulation of peroxisomal lipid metabolism, and/or (2) DEHP-altered hepatic distribution of the TCDD.

In another study evaluating the effect of DEHP on the peroxisomal system, Perera et al. (1986) reported increased effects in rats kept on a choline-deficient diet. This conclusion was based on an increase in the conjugated dienes (indicators of free radical oxygen modification of cellular lipids) in the microsomes of choline-deficient animals exposed to 500 mg/kg DEHP for 4 weeks.

Other studies have indicated potential additive effects regarding liver toxicity with DEHP and other chemicals. In a full-factorial study evaluating potential interactions between DEHP, trichloroethylene, and heptachlor, with respect to systemic toxicity, the study authors reported a greater-than-additive effect on liver toxicity between DEHP and trichloroethylene (Simmons et al. 2005). However, this study was only available as an abstract, and conclusions cannot be independently reviewed. Another study evaluated hepatic endpoints in male rats following dietary exposure to 10,000 ppm DEHP, 10,000 ppm di-*n*-hexyl phthalate (DnHP), or their combination (Howarth et al. 2001). These study authors indicated that decreases in serum cholesterol “seemed additive” for the mixture, while all other hepatic effects observed in DEHP+DnHP-treated animals were similar to those observed in DEHP-treated animals. However, the study design was inadequate to evaluate interactions due to lack of dose-response data for individual chemicals or mixture.

Several hepatic endpoints were evaluated in male rats in the 4-week study by Dalgaard et al. (2000) evaluating potential interactions between DEHP and acetone described in the male reproductive section

above, including clinical chemistry, liver weight, and liver histology. No significant interactions were observed with respect to any of these endpoints.

Toxicokinetic Interactions. Co-exposure to the food emulsifier, glycerin monostearate, increased the oral absorption of DEHP when co-administered to rats (Gao et al. 2016). This increase in bioavailability resulted in an increase in DEHP-induced male reproductive toxicity (decreased testosterone, sperm damage) in rats co-exposed to DEHP and glycerin monostearate compared with exposure to DEHP alone (Gao et al. 2016).

In studies of the effects of DEHP ingestion on the metabolism of ethanol, there was a distinct difference between the action of single doses of 1,500–7,500 mg/kg DEHP and the same doses given over a 7-day period (Agarwal et al. 1982). The single dose appeared to decrease the metabolism of intraperitoneal ethanol, given 18 hours after DEHP, as reflected by an increase in the ethanol-induced sleeping time of the exposed rats and inhibition of hepatic alcohol dehydrogenase activity. On the other hand, when DEHP was given for 7 days before the ethanol, the ethanol-induced sleeping time was decreased and the activities of both alcohol and aldehyde dehydrogenase were increased. This indicates that the changes in sleeping time were the result of more rapid metabolic removal of the alcohol from the system in the rats treated with repeated doses of DEHP and slower metabolism in the rats given one dose.

Companion *in vitro* studies of the effects of DEHP, MEHP, and 2-ethylhexanol on the activities of alcohol and aldehyde dehydrogenase indicated that it is the metabolites of DEHP that affect the enzymes, rather than unmetabolized DEHP (Agarwal et al. 1982). The authors suggested that 2-ethylhexanol acts as a competitive inhibitor of alcohol dehydrogenase when a single dose of DEHP is administered. When DEHP exposure occurred for several days prior to ethanol exposure, the liver adjusted to the metabolic demands of the 2-ethylhexanol. Thus, at the time of ethanol ingestion, most of the 2-ethylhexanol was metabolized and the capacity of the liver to metabolize the ethanol was expanded due to the induction of the alcohol-metabolizing enzymes.

Other Interactions. In the 4-week study by Dalgaard et al. (2000) evaluating potential interactions between DEHP and acetone described in the male reproductive section above, an apparent increase in DEHP-associated lethality at the highest dose (10,000 mg/kg/day) was observed with co-exposure to acetone for 4 weeks. Observed mortality was 2/10 in the DEHP-only group and 4/10 in the DEHP+acetone group.

One study evaluated potential interactions between DEHP and benzo(a)pyrene (BaP) with respect to female reproductive toxicity (Xu et al. 2010). Female XX rats were exposed to DEHP at 300 or 600 mg/kg/day, BaP at 5 or 10 mg/kg/day, or a combination of the low- or high-doses of each for 60 days via gavage (every other day). Examined endpoints include ovary weight, estrous cycle, serum hormone levels, ovarian follicle populations, granulosa cell apoptosis, and gene and protein expression of aromatase and PPAR. While both chemicals caused exposure-related changes in certain outcomes, there was no qualitative evidence of interaction (no formal statistical interaction analysis was conducted).

Intermediate-duration oral studies in rats have shown that high doses of DEHP can affect thyroid cell structure (e.g., hypertrophy of Golgi apparatus, increases in lysosomes, dilation of the endoplasmic reticula, and increases in colloid droplets) and function (e.g., decreased levels of circulating T4) (Hinton et al. 1986; Poon et al. 1997; Price et al. 1987, 1988). When large oral doses of 500 and 2,500 mg/kg/day DEHP were combined with dietary exposure to a compound that has similar effects on the thyroid (Aroclor 1254, a polychlorinated biphenyl mixture), there was an apparent additive effect of the two compounds on changes in thyroid cell structure and decreases in serum T3 and T4. At lower doses of DEHP (50 and 100 mg/kg/day) and Aroclor 1254, there were no additive effects apparent with the changes in cell structure or the levels of T3 and T4. In another study, Howarth et al. (2001) did not observe any interaction between DEHP and DnHP with regard to thyroid toxicity in male rats following dietary exposure to 10,000 ppm DEHP, 10,000 ppm DnHP, or their combination for 14 days; however, the study design was inadequate to evaluate interactions due to lack of dose-response data for individual chemicals or mixture.