3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of DEHP. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

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

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. Dose conversions, if necessary, were performed using EPA reference methodology. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health

effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of DEHP are indicated in Table 3-2 and Figure 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for DEHP. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990e), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs. A limited amount of information is available on health effects of DEHP in humans and essentially all of the significant levels of exposure that are presented in Tables 3-1 and 3-2 and Figures 3-1 and 3-2 are based on animal data. The LOAELs observed in animal studies cannot be directly extrapolated to humans, do not necessarily constitute levels of concern for humans, and are much higher than intake levels normally encountered by humans. As discussed in Chapter 6 (Section 6.5, Potential for Human Exposure), recent estimates of average total daily intake of DEHP from ambient exposures in the U.S. general population are in the range of 3–30 Fg/kg/day (David 2000; Doull et al. 1996; Huber et al. 1996; Kohn et al. 2000; NTP 2000b; Tickner et al. 2001), which is 3–4 orders of magnitude lower than the lowest LOAELs in animals. Additional factors that may preclude the direct extrapolation or assumption of similar effects in animals and humans include the dose-related, species-specific, and route-specific nature of some of the effects, such as evidence indicating that most of the hepatic changes observed in DEHP-exposed rodents, including liver cancer, result from a mechanism that does not operate in humans (see Section 3.5, Mechanisms of Action).

3.2.1 Inhalation Exposure

3.2.1.1 Death

No studies were located regarding lethality in humans after inhalation exposure to DEHP.

Studies in animals suggest that DEHP has low toxicity when inhaled. No deaths occurred in rats exposed to 300 mg/m^3 for 6 hours/day for 10 days (Merkle et al. 1988) or hamsters exposed to 0.015 mg/m^3 for their lifetime (Schmezer et al. 1988). At a concentration of 0.015 mg/m^3, DEHP is present as a vapor, while at 300 mg/m^3 it is an ultra fine aerosol. On the other hand, DEHP was found to be lethal to rats after 2–4 hours of exposure to a mist prepared by passing air through a heated sample of DEHP (Shaffer et al. 1945). The concentration of DEHP in the mist was not measured.

3.2.1.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, musculoskeletal, renal, or dermal/ocular effects in humans or animals after inhalation exposure to DEHP. The systemic effects observed after inhalation exposure are discussed below. The highest NOAEL and all reliable LOAEL

values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-1 and Figure 3-1.

Respiratory Effects. Unusual lung effects were observed during the fourth week of life in three children who were exposed to DEHP during mechanical ventilation as preterm infants (Roth et al. 1988). The effects clinically and radiologically resembled hyaline membrane disease, a disorder caused by insufficient surfactant production in the lungs of newborn infants. Although interpretation of these findings is complicated by the preexisting compromised health status of the preterm infants, the information indicates that the lung disorders were related to DEHP released from the walls of polyvinyl chloride (PVC) respiratory tubes.

Increased lung weights accompanied by thickening of the alveolar septa and proliferation of foam cells were observed in male rats that were exposed to 1,000 mg/m^3 of DEHP aerosol for 6 hours/day, 5 days/week for 4 weeks (Klimisch et al. 1991). These effects were reversible within an 8-week post-exposure period. Females rats exposed to this concentration were not affected nor were animals of either sex at concentrations of 10 and 50 mg/m^3.

Hepatic Effects. No studies were located regarding hepatic effects in humans after inhalation exposure to DEHP. In male and female rats relative liver weights were increased by exposure to 1,000 mg/m^3 administered as an aerosol 6 hours/day, 5 days/week for a 4-week period (Klimisch et al. 1991). However, there was no evidence of peroxisome proliferation in thin slices of the livers examined under an electron microscope. Relative liver weights were not increased in animals examined 8 weeks after the last exposure to DEHP.

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to DEHP.

Table 3-1. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL		Reference Chemical Form
					Less serious (mg/m3)	Serious (mg/m3)	
ACUTE EXPOSURE							
Developmental							
1	Rat (Wistar)	10 d Gd 6-15 6hr/d		300			Merkle et al. 1988

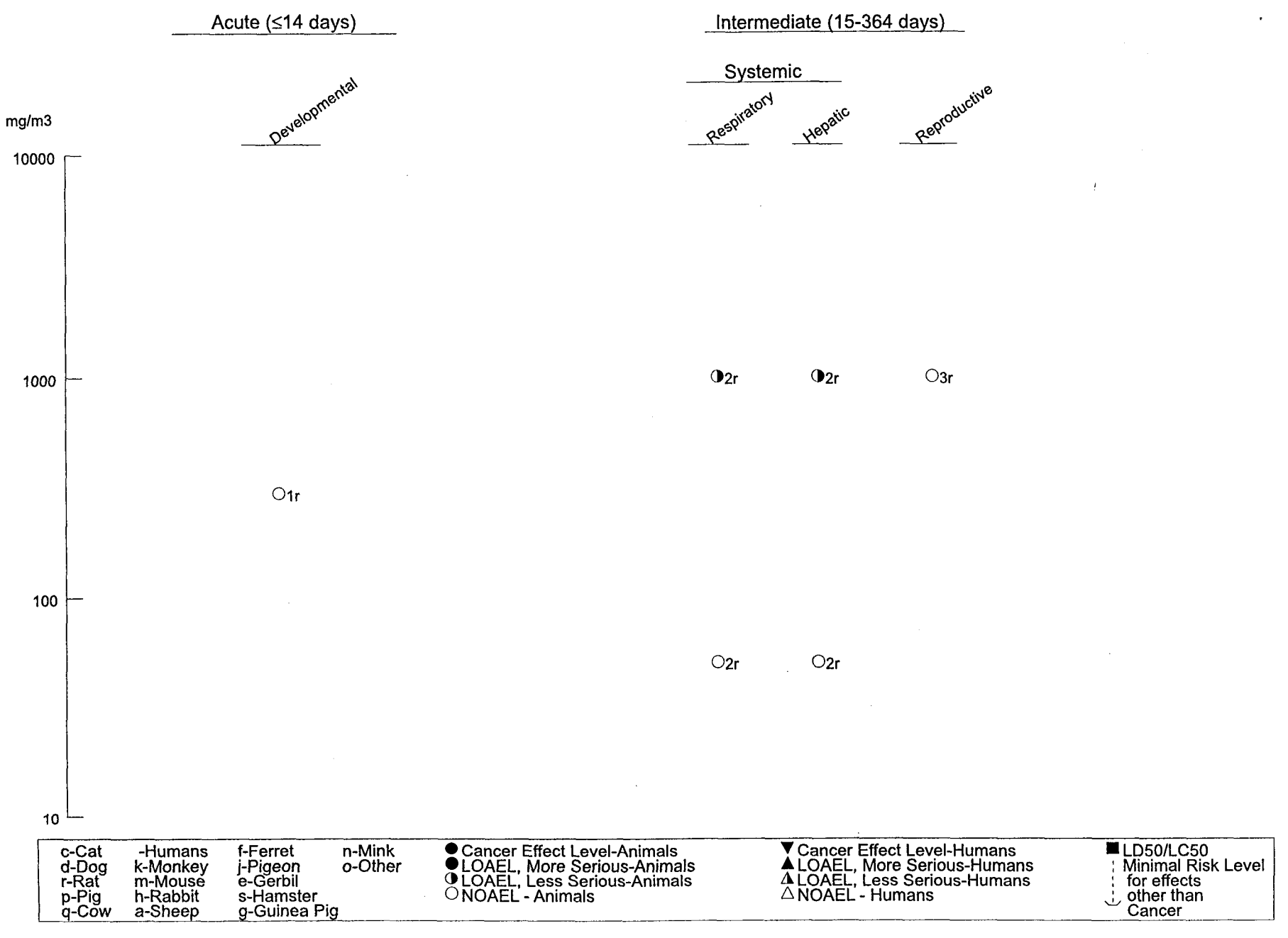
Table 3-1. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
Systemic							
2	Rat (Wistar)	28 d 5 d/wk 6 hr/d	Resp	50	1000 (increased lung weight, foam cell proliferation, thickening of alveolar septa)		Klimisch et al. 1991
			Hepatic	50	1000 (increased relative liver weight)		
Reproductive							
3	Rat (Wistar)	28 d 5 d/wk 6 hr/d		1000			Klimisch et al. 1991

[a]The number corresponds to entries in Figure 3-1.

d = days; Gd = gestation day; hr = hour(s); LOAEL = lowest-observed-effect level; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Figure 3-1. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Inhalation



3.2.1.4 Neurological Effects

No studies were located regarding neurological effects in humans or animals after inhalation exposure to DEHP.

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to DEHP. The fertility and mating performance of male rats was not effected by a 4-week exposure, 6 hours/day, 5 days/week to a DEHP aerosol (10–1,000 mg/m^3) (Klimisch et al. 1991). Mating with unexposed females was carried out at 2 and 6 weeks after the end of DEHP exposure period. At sacrifice, there were no observable effects of DEHP on testicular structure.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation exposure to DEHP. In rats, there was no evidence of any treatment-related prenatal or postnatal developmental effects in the offspring of females exposed to up to 300 mg/m^3 DEHP (the highest dose tested) 6 hours/day during the period of organogenesis (gestation days 6–15) (Merkle et al. 1988). Newborn rats were evaluated for survival and several measures of neurological development (righting test on day 6, gripping reflex on day 13, pupillar reflex on day 20, and hearing test on day 21). These data indicated that there were no developmental effects when DEHP was present in the atmosphere during gestation. This NOAEL for developmental effects in rats is recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.7 Cancer

No studies were located regarding cancer in humans after inhalation exposure to DEHP. Lifetime exposure of hamsters to 0.015 mg/m^3 DEHP did not result in any significant increases in the incidence of tumors (Schmezer et al. 1988). Because the concentration in this study was very low, it is not possible to reach conclusions concerning whether or not higher concentrations might produce different results.

3.2.2 Oral Exposure

3.2.2.1 Death

Single oral doses of up to 10 g DEHP are not lethal to humans (Shaffer et al. 1945), and no cases of death in humans after oral exposure to DEHP were located. These data indicate that DEHP is very unlikely to cause acute mortality in humans. This is supported by studies in rabbits and rats which indicate that single dose oral LD_{50} values are quite high (30,600–33,900 mg/kg) (Shaffer et al. 1945). To receive an equivalent dose, an adult human would have to consume about 4½ pounds of DEHP. Some species seem to be more sensitive than others. Repeated administrations of 2,000 mg/kg/day DEHP (the only dose tested) was lethal to adult rabbits and guinea pigs when administered for up to 7 days, but not to adult mice and rats (Parmar et al. 1988). However, two doses of 2,000 mg DEHP/kg caused a high incidence of mortality in #21-day-old rats, but there were no deaths in 6-week-old or older rats, suggesting that age influences susceptibility to DEHP (Dostal et al. 1987a). Similar results regarding higher susceptibility to lethal doses among younger rats were reported by Parmar et al. (1994). Treatment of lactating female rats (postpartum days 1–7) with 5,000 mg DEHP/kg by gavage resulted in 25% mortality within 1 week of treatment, but no mortality occurred with #2,500 mg DEHP/kg (Cimini et al. 1994). In a 24-week feeding study, a diet that provided approximately 2,400 mg DEHP/kg/day induced 100% mortality in Sv/129 male mice after 16 weeks of dosing (Ward et al. 1998); at the time of death, mean body weights were approximately 50% that of controls, food consumption data were not provided. Survival was reduced in male F344 rats (12% less than controls) and male B6C3F1 mice (45% less than controls) that ingested 147 and 1,266 mg DEHP/kg/day in the diet, respectively, for up to 104 weeks (David et al. 2000a, 2000b). The most frequent cause of death in the chronic studies was mononuclear cell leukemia in the rats and liver tumors in the mice. The LOAEL values for death in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE						
	Death						
1	Rat (Fischer- 344)	7 d ppd 1-7 1 x/d (GO)				5000 F (25% mortality within one week)	Cimini et al. 1994
2	Rat (Sprague-Dawley)	5 d 1 x/d (GO)				1000 (68% mortality in 14-18-day-old rats died after 5 doses; older rats were less susceptible)	Dostal et al. 1987a
3	Rat (Wistar)	7 d 1 x/d (GO)				2000 (10% mortality at 7 days in 3-week-old rats; 0% in untreated or in treated older rats)	Parmar et al. 1994
4	Rat (Wistar)	1 d 1x/d (G)				30600 (LD_{50})	Shaffer et al. 1945
5	Rabbit (NS)	7 d 1x/d (GO)				2000 (50% mortality)	Parmar et al. 1988
6	Rabbit (NS)	1 d 1x/d (G)				33900 (LD_{50})	Shaffer et al. 1945
	Systemic						
7	Human	1 dose 1x/d (C)	Gastro	71.4	143 (gastrointestinal distress)		Shaffer et al. 1945

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
8	Monkey (Cynomolgus)	14 d 1 x/d (G)	Hemato	500 M			Pugh et al. 2000
			Hepatic	500			
			Renal	500			
			Bd Wt	500			
9	Monkey (Marmoset)	14 d 1x/d (GO)	Hepatic		2000 (20% increase in liver weight)		Rhodes et al. 1986
			Renal	2000			
			Bd Wt			2000 (70% reduction in body weight gain)	
10	Rat (Fischer- 344)	3 d 1 x/d (GO)	Hepatic		1200 (altered liver lipid profile)		Adinehzadeh and Reo 1998
			Other		1200 (44% reduction in food consumption)		
11	Rat (Fischer- 344)	once (GO)	Hepatic		1500 (centrilobular necrosis or inflammation)		Berman et al. 1995
			Endocr	5000			
12	Rat (Fischer- 344)	14 d 1 x/d (GO)	Hepatic		150 (18% increase in relative liver weight; incresed metosis)		Berman et al. 1995
			Endocr	1500			
13	Rat (Fischer- 344)	7 d (F)	Hepatic	11	53 M (increased relative liver weight)		David et al. 1999

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)	Reference Chemical Form
14	Rat (Sprague-Dawley)	5 d 1x/d (GO)	Hepatic	10	100	(increased liver weight and activity of palmitoyl CoA oxidase and carnitine acetyl transferase)		Dostal et al. 1987a
			Renal	100	1000	(increased kidney weight)		
15	Rat (Sprague-Dawley)	5 d Ld 6-10 1x/d (GO)	Hepatic		2000	(increased liver weight; increased enzyme activity; decreased serum cholesterol)		Dostal et al.1987b
			Bd Wt		2000	(18% decrease in body weight gain)		
16	Rat (Sprague-Dawley)	5 d Ld 14-18 1x/d (GO)	Hepatic		2000	(increased liver weights and enzyme activities; decreased cholesterol and triglycerides)		Dostal et al. 1987b
			Bd Wt		2000	(17% decrease in bd wt gain)		
			Other		2000	(decreased food consumption)		
17	Rat (Sprague-Dawley)	5 d Ld 2-6 1x/d (GO)	Hepatic		2000	(increased liver weight; increased enzyme activity; decreased plasma cholesterol and triglycerides)		Dostal et al. 1987b
			Bd Wt		2000	(14% decrease in body weight gain)		
18	Rat (Sprague-Dawley)	5 d 1x/d (GO)	Bd Wt	500	1000	(15% decrease in body weight gain)		Dostal et al. 1988

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less serious (mg/kg/day)		Serious (mg/kg/day)		
19	Rat (Wistar)	10 d ppd 105-114 1x/d (GO)	Bd Wt		2800	(11% decrease in final body weight)			Gray and Butterworth 1980
20	Rat (Wistar)	10 d ppd 70-77 1x/d (GO)	Bd Wt				2800	(21% reduction in final body weight)	Gray and Butterworth 1980
21	Rat (Wistar)	10 d ppd 28-37 1x/d (GO)	Bd Wt				2800	(22% reduction in final body weight)	Gray and Butterworth 1980
22	Rat (Wistar)	3 d (F)	Endocr		2000	(decreased serum T4 ultrastructural changes consistent with thyroid hyperactivity)			Hinton et al. 1986
23	Rat (Sprague-Dawley)	7 d (F)	Hepatic		2000	(35% increase in relative liver weight; induction of microsomal carboxylesterases)			Hosokawa et al. 1994
24	Rat (Fischer- 344)	2 d 1 x/d (GO)	Hepatic		950	(26% increase in absolute liver weight; 1300% increased DNA synthesis; 20% reduced apoptosis)			James et al. 1998
			Bd Wt	950					

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
25	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Hepatic		1000 (72 % increased relative liver weight; increased activity of peroxisomal and microsomal enzymes)		Lake et al. 1986
26	Rat (Sprague-Dawley)	10 d (F)	Bd Wt			1740 M (22% lower final body weight)	Mehrotra et al. 1997
27	Rat (Wistar)	7 d 1x/d (GO)	Hepatic		1000 (36% increased relative liver weight)		Oishi 1989
			Bd Wt	1500			
28	Rat (Wistar)	7 d 1 x/d (GO)	Hepatic		2000 (52% increased relative liver weight)		Oishi 1994
29	Rat (albino)	7 d 1x/d (GO)	Hepatic		2000 (increased liver weight, increased enzyme activity)		Parmar et al. 1988
			Bd Wt		2000 (10% decrease in body weight gain)		
30	Rat (Wistar)	14 d 1x/d (GO)	Hepatic		2000 (40% increase in liver weight, peroxisome proliferation)		Rhodes et al. 1986
			Renal	2000			
			Bd Wt			2000 (40% decrease in body weight gain)	

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
31	Rat (Sprague-Dawley)	14d (F)	Hepatic		1905	(87% increased liver weight; peroxisome proliferation; increased synthesis of NAD^+ from tryptophan)			Shin et al. 1999
			Bd Wt	1905					
32	Rat (Sprague-Dawley)	14 d ppd 25-38 (F)	Bd Wt		1000	(22% decreased body weight gain)			Sjoberg et al. 1986a
33	Rat (Sprague-Dawley)	14 d ppd 40-53 (F)	Bd Wt		1700	(22% decreased body weight gain)			Sjoberg et al. 1986a
34	Rat (Sprague-Dawley)	14 d ppd 60-73 (F)	Bd Wt		1000	(26% decreased body weight gain)			Sjoberg et al. 1986a
35	Rat (Sprague-Dawley)	14 d (F)	Bd Wt				1000	(22% reduction in final body weight)	Sjoberg et al. 1986b
36	Rat (Fischer- 344)	14 d (F)	Hepatic		1200	(increased liver weight, increased oxidized deoxyguanosine in DNA)			Takagi et al. 1990
			Renal		1200	(increased kidney weight)			
37	Rat (Wistar)	14 d (F)	Hepatic		1894	(38% increased absolute liver weight; peroxisomal proliferation)			Van den Munckhof et al. 1998
			Bd Wt		1894	(17% reduction in final body weight)			

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
38	Mouse (B6C3F1)	7d (F)	Hepatic	188	564	(increased relative liver weight)			David et al. 1999
39	Mouse (C57BL/6)	7 d (F)	Hepatic		4000	(88% increase in relative liver weight; induction of microsomal carboxylesterases)			Hosokawa et al. 1994
40	Mouse (B6C3F1)	2 d 1 x/d (GO)	Hepatic		1150	(9% increase in absolute liver weight; 248% increased DNA synthesis; 90% decreased apoptosis)			James et al. 1998
			Bd Wt	1150					
41	Mouse (C57BL/6)	7 d (F)	Hepatic		385	(increased absolute and relative liver weight)			Muhlenkamp and Gill 1998
			Bd Wt		3850	(17% decrease in final body weight)			
42	Mouse (NS)	7 d 1x/d (GO)	Hepatic		2000	(increased liver weight, increased enzyme activity)			Parmar et al. 1988
			Bd Wt	2000					
43	Gn Pig (NS)	7 d 1x/d (GO)	Hepatic		2000	(increased liver weight, increased enzyme activity)			Parmar et al. 1988
			Bd Wt				2000	(39% decrease in body weight gain)	
44	Hamster (Golden Syrian)	7 d (F)	Hepatic		2686	(36% increase in relative liver weight)			Hosokawa et al. 1994

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
45	Hamster Chinese	14 d 1x/d (GO)	Hepatic		1000 (55% increased liver weight, enzyme induction)		Lake et al. 1986
46	Rabbit (NS)	7 d 1x/d (GO)	Hepatic		2000 (decreased liver weight, decreased enzyme activity)		Parmar et al. 1988
			Bd Wt	2000			
	Immunological/Lymphoreticular						
47	Rat (Fischer- 344)	once (GO)		5000			Berman et al. 1995
48	Rat (Fischer- 344)	14 d 1 x/d (GO)		1500			Berman et al. 1995
	Neurological						
49	Rat (Fischer- 344)	once (GO)		1500	5000 (signs of general debilitation)		Moser et al. 1995
50	Rat (Fischer- 344)	14 d 1 x/d (GO)		1500			Moser et al. 1995
	Reproductive						
51	Monkey (Cynomolgus)	14 d 1 x/d (G)		500 M			Pugh et al. 2000
52	Monkey (Marmoset)	14 d 1x/d (GO)		2000			Rhodes et al. 1986

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
53	Rat (Sprague-Dawley)	1-10 d 1x/d (GO)				2000 F (suppressed ovulation with 25% decrease in preovulatory follicle granulosa cells and decresed serum estradiol)	Davis et al. 1994a
54	Rat (Sprague-Dawley)	3 d Ld 15-17 1x/d (GO)			2000 (changes in milk composition)		Dostal et al. 1987b
55	Rat (Sprague-Dawley)	5 d ppd 6-10 1x/d (GO)		100	1000 (reduced absolute and relative testes weight and number of Sertoli cells)		Dostal et al. 1988
56	Rat (Sprague-Dawley)	5 d ppd 86-90 1x/d (GO)		100	1000 (loss of spermatids and spermatocytes; decreased testicular zinc)		Dostal et al. 1988
57	Rat (Sprague-Dawley)	5 d ppd 6-10 1x/d (GO)		100	200 (reduced testicular weight, delayed spermatid maturation 4 weeks after dosing)		Dostal et al. 1988
58	Rat (Sprague-Dawley)	5 d ppd 6-10 1x/d (GO)		200	500 (reduced relative testes weight and number of Sertoli cells)		Dostal et al. 1988
59	Rat (Sprague-Dawley)	10 d ppd 105-114 1x/d (GO)		2800			Gray and Butterworth 1980

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
60	Rat (Sprague-Dawley)	10 d ppd 70-79 1x/d (GO)			2800	(moderate testicular damage; decrease in seminal vesicle prostate weight)			Gray and Butterworth 1980
61	Rat (Wistar)	10 d ppd 70-79 1x/d (GO)			2800	(decreased weight of seminal vesicles and ventral prostate; tubular damage)			Gray and Butterworth 1980
62	Rat (Wistar)	10 d ppd 28-37 1x/d (GO)					2800	(33% decreased relative testes weight; loss of germinal cells; decrease in seminal vesicle and ventral prostate weight)	Gray and Butterworth 1980
63	Rat (Sprague-Dawley)	10 d ppd 28-37 1x/d (GO)					2800	(47% decreased testes weight; severe testicular atrophy)	Gray and Butterworth 1980
64	Rat (Sprague-Dawley)	1 d Gd 3 (GO)		20 M	100 M	(abnormal gonocytes and reduced Sertoli cell proliferation)			Li et al. 2000
65	Rat (Sprague-Dawley)	10 d (F)			1740 M	(25-50% changes in testicular xenobiotic enzyme activity)			Mehrotra et al. 1997
66	Rat (Wistar)	10 d ppd 30-39 1x/d (G)			2000	(aspermatogenesis with reduced testis, seminal vesicle, and ventral prostrate weights; decreased testicular zinc)			Oishi 1986

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less serious (mg/kg/day)		Serious (mg/kg/day)		
67	Rat (Wistar)	7 d 1 x/d (GO)					2000	(38% reduced testis weight; shrunken seminiferous tubules with necrotic debris and aspermatogenesis)	Oishi 1994
68	Rat (Sprague-Dawley)	Once (GO)			2800	(morphological changes in Sertoli cells)			Saitoh et al. 1997
69	Rat (Sprague-Dawley)	14 d ppd 25-38 1x/d (GO)			1000	(testicular damage)			Sjoberg et al. 1986a
70	Rat (Sprague-Dawley)	14 d ppd 60-73 (F)		1700					Sjoberg et al. 1986a
71	Rat (Sprague-Dawley)	14 d ppd 40-53 (F)		1000			1700	(43% decreased testicular weight and severe seminiferous tubule damage)	Sjoberg et al. 1986a
72	Rat (Sprague-Dawley)	14 d ppd 40-53 1x/d (GO)		1000					Sjoberg et al. 1986a
73	Rat (Sprague-Dawley)	14 d ppd 60-73 1x/d (GO)		1000					Sjoberg et al. 1986a
74	Rat (Sprague-Dawley)	14 d ppd 25-38 (F)			1000	(21% decreased testicular weight and tubular damage)	1700	(79% decreased testicular weight and severe testicular damage)	Sjoberg et al. 1986a

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
75	Rat (Sprague-Dawley)	4 d 1x/d (GO)		2000			Zacharewski et al. 1998
	Developmental						
76	Rat (Sprague-Dawley)	5 d Ld 14-18 1x/d (GO)			2000 (14% reduction in pup body weight; biochemical evidence of peroxisome proliferation in liver)		Dostal et al. 1987b
77	Rat (Sprague-Dawley)	5 d Ld 2-6 1x/d (GO)			2000 (26% reduction in pup body weight; biochemical evidence of peroxisome proliferation in liver)		Dostal et al. 1987b
78	Rat (Sprague-Dawley)	5 d Ld 6-10 1x/d (GO)			2000 (20% reduction in pup body weight; biochemical evidence of peroxisome proliferation in liver)		Dostal et al. 1987b
79	Rat (Sprague-Dawley)	10 d Gd 14-21 ppd 1-3 1 x/d (GO)				750 (significant delay in male reproductive system maturation; reduced weight of sex organs in adult males)	Gray et al. 1999
80	Rat (Sprague-Dawley)	10 d Gd 14-21 ppd 1-3 1 x/d (GO)				750 M (testicular degeneration and altered sexual differentiation in male offspring)	Gray et al. 2000

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less serious (mg/kg/day)		Serious (mg/kg/day)		
81	Rat (Wistar)	9 d Gd 6-15 1 x/d (GO)		200			1000	(increased fetal death and incidence of external, soft tissue, and skeletal malformations)	Hellwig et al. 1997
82	Rat (Sprague-Dawley)	10 d Gd 14-21 ppd 1-3 1 x/d (GO)					750 M	(decreased fetal testosterone synthesis during male sexual differentiation)	Parks et al. 2000
83	Rat (Wistar)	1 d Gd 12 1x/d (G)			4882	(slight increase in dead, resorbed and malformed fetuses)	9756	(significant increase in dead, resorbed and malformed fetuses)	Ritter et al. 1987
84	Rat (Fischer- 344)	14d ppd 1-21 1 x/d (GO)			1000	(significant peroxisome proliferation in both liver and kidneys from pups)			Stefanini et al. 1995
85	Mouse (C57BL/6N x Sv/129)	2 d Gd 8-9 1 x/d (GO)					1000	(decreased fetal viability, increased resorptions and external malformations)	Peters et al. 1997b
86	Mouse (Slc-ICR)	3 d Gd 7-9 1 x/d (GO)		250			1000	(decreased fetal viability, increased resorptions and external malformations)	Shiota and Mima 1985
87	Mouse (ddY-Slc)	Gd 6, 7, 8, 9, or 10 1 x/d (G)		50			100	(11.2 % fetal lethality, 2.0% in controls)	Tomita et al. 1982a

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)		
88	Mouse (ddY-Slc)	Gd 6, 7, 8, 9, or 10 1 x/d (G)				1000	(60% fetal lethality)	Yagi et al. 1980

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
	INTERMEDIATE EXPOSURE							
	Death							
89	Rat (Wistar)	15 d (GO)				2000	(50% mortality after 3 weeks with subsequent 100% mortality)	Parmar et al. 1987
90	Mouse Sv/129	16 wk (F)				2400 M	(100% mortality between weeks 12 and 16)	Ward et al. 1998
91	Gn Pig (NS)	15 d (GO)				2000	(40% mortality)	Parmar et al. 1988
92	Rabbit (NS)	15 d (GO)				2000	(100% mortality)	Parmar et al. 1988
	Systemic							
93	Monkey (Marmoset)	13 wk 1x/d (GO)	Resp	2500				Kurata et al. 1998
			Cardio	2500				
			Gastro	2500				
			Hemato	2500				
			Musc/skel	2500				
			Hepatic	2500				
			Renal	2500				
			Endocr	2500				
			Dermal	2500				
			Ocular	2500				
94	Monkey (Cynomolgus)	25 d 1x/d (GO)	Hepatic	500				Short et al. 1987

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
95	Rat (Fischer- 344)	21 d (F)	Hepatic	12	643	(44% increase in relative liver weight; increased enzymatic activity indicative of peroxisome proliferation)			Barber et al. 1987
			Bd Wt	1197			1892	(41% reduced final body weight)	
96	Rat (Fischer- 344)	28 d (F)	Hepatic		1200	(increased enzyme activities indicating peroxisome proliferation)			Cattley et al. 1988
97	Rat (Fischer- 344)	2-13 wk (F)	Hepatic	53	265	(increased relative liver weight)			David et al. 1999
98	Rat (Fischer- 344)	4-16 wk (F)	Hepatic		1054	(increased relative liver weight and biochemical evidence of cell proliferation)			Eagon et al. 1994
			Endocr		1054	(altered metabolism of estradiol and estrogen receptor related functions)			
			Bd Wt		1054	(19% reduction in final body weight relative to controls at 4 weeks)			
99	Rat (Alpk/AP)	28 d 1x/d (GO)	Hepatic		1000	(increased palmitoyl-CoA oxidase activity; decreased superoxide dismutase and glutathione peroxidase activities)			Elliot and Elcombe 1987

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
100	Rat (Wistar)	21 d (F)	Endocr		2000	(decreased serum T4 ultrastructural changes consistent with thyroid hyperactivity)			Hinton et al. 1986
101	Rat (Fischer- 344)	28 d (F)	Hepatic		705	(>53% increase in relative liver weights; morphological and biochemical evidence of peroxisome proliferation)			Hodgson 1987
			Bd Wt	705					
102	Rat (albino)	9 mo (F)	Hepatic		50	(increased liver weight, morphological changes in bile ducts, lipid filled lysosomes, glycogen depletion, induction of peroxisomal enzymes and cytochrome P-450 system)			Mitchell et al. 1985b
			Bd Wt	50	200	(10-15% decreased body weight gain)			
103	Rat (Wistar)	21 d (F)	Hepatic		1730	(41% increased absolute liver weight)			Mocchiutti and Bernal 1997
			Bd Wt				1730	(28% reduction in final body weight)	
104	Rat (albino)	15 d 1x/d (GO)	Hepatic		2000	(increased liver weight, changes in enzyme activity)			Parmar et al. 1988
			Bd Wt				2000	(24% decrease in body weight gain)	

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less serious (mg/kg/day)		Serious (mg/kg/day)	
105	Rat (Sprague-Dawley)	13 wk (F)	Hemato	37.6 M	375 M	(decreased RBCs and hemoglobin)		Poon et al. 1997
			Hepatic	37.6 M	375 M	(increased absolute and relative liver weights; peroxisome proliferation)		
			Renal	37.6 M	375 M	(increased kidney weight)		
			Bd Wt	375 M				
			Other	375 M				
106	Rat (Sprague-Dawley)	13 wk (F)	Hemato		345 M	(increased platelet count)		Poon et al. 1997
			Hepatic		345 M	(increased absolute and relative liver weights; peroxisome proliferation)		
			Renal		345 M	(increased kidney weight)		
			Endocr		345 M	(reduced follicle size and colloid density in the thyroid)		
107	Rat (Wistar)	3 mo (F)	Endocr		1000	(ultrastructural changes consistent with thyroid hyperactivity)		Price et al. 1988a
108	Rat (Wistar)	90 d (F)	Cardio	1900				Shaffer et al. 1945
			Hemato	1900				
			Hepatic	1900				
			Renal	1900				
			Bd Wt	200	400	(decreased weight gain)		

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
109	Rat (Fischer- 344)	21 d (F)	Hepatic	11	105	(biochemical and morphological evidence of peroxisome proliferation)			Short et al. 1987
			Bd Wt				2100	(no weight gain)	
110	Rat (Fischer- 344)	54 d 1x/d (GO)	Hepatic		2000	(89% increase in relative liver weight; peroxisome proliferation)			Tomaszewski et al. 1988
111	Rat (Fischer- 344)	20 d Gd 0-20 (F)	Hepatic		357	(increased relative liver weights)			Tyl et al. 1988
			Bd Wt	357	666	(19% reduced maternal weight gain)	856	(39% reduced maternal weight gain)	
112	Mouse (B6C3F1)	4-13 wk (F)	Hepatic		188 F	(increased relative liver weight)			David et al. 1999
113	Mouse (Cr1:CD-1)	126 d (F)	Hepatic		420	(increased liver weights)			Lamb et al. 1987
			Bd Wt	420					
114	Mouse (ICR)	4 wk 2 d/wk (GO)	Bd Wt	1171					Lee et al. 1997
115	Mouse (NS)	15 d 1x/d (GO)	Hepatic		2000	(increased liver weight, changes in enzyme activity)			Parmar et al. 1988
			Bd Wt		2000	(11% change in body weight gain)			

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
116	Mouse (CD-1)	17 d Gd0-17 (F)	Other	44	91 (rough coat; lethargy)		Tyl et al 1988
			Hepatic	91	191 (increased relative liver weight)		
			Bd Wt	91		191 (30% reduced weight gain)	
117	Mouse Sv/129	24 wk (F)	Hepatic			2400 M (degenerative liver lesions)	Ward et al. 1998
			Renal			2400 M (degenerative renal lesions)	
			Bd Wt			2400 M (50% lower final body weight)	
118	Mouse (CH3/HeNCr)	24 wk (F)	Hepatic		1953 (significant increase in relative liver weight)		Weghorst et al. 1994
			Bd Wt			1953 (final body weight reduced over 50%)	
119	Gn Pig (NS)	15 d 1x/d (GO)	Hepatic		2000 (increased liver weight, decreased enzyme activity)		Parmar et al. 1988
			Bd Wt		2000 (19% decrease in body weight gain)		
120	Hamster (Golden Syrian)	30 wk (F)	Hepatic	1436			Maruyama et al. 1994
			Renal		1436 (increase relative kidney weight)		
			Bd Wt		1436 (16% reduction in final body weight)		
	Immunological/Lymphoreticular						
121	Rat (Wistar)	90 d (F)		1900 M			Shaffer et al. 1945

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Reproductive						
122	Monkey (Marmoset)	13 wk 1x/d (GO)		2500 M			Kurata et al. 1998
123	Rat (Wistar)	42 d (F)			1200 (decreased testicular weight, seminal vesilce and ventral prostrate with gradual post-exposure recovery)		Gray and Butterworth 1980
124	Rat (Sprague-Dawley)	40 d Gd 3-21 ppd 1-21 (GO)				375 M (altered sexual differentiation and decreased testes and anterior prostate weights in male offspring)	Moore et al. 2001
125	Rat (Wistar)	15 d (GO)				2000 (decreased testicular weight, changes in tubules, damaged spermatogenic cells; reduced sperm count)	Parmar et al. 1987
126	Rat (Wistar)	30 d 1x/d (GO)			50 M (33% lower testicular weight)	250 M (57% lower testicular weight, testicular germ cell damage)	Parmar et al. 1995
127	Rat (Sprague-Dawley)	13 wk (F)		3.7 M	37.6 M (mild vacuolation of Sertoli cells in 7/10 rats)	375.2 M (testicular atrophy with complete loss of spermatogenesis in 9/10 rats)	Poon et al. 1997
128	Rat (Sprague-Dawley)	13 wk 1x/d (F)				345 M (testicular atrophy)	Poon et al. 1997

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
129	Rat (Wistar)	126 wk (F)		339 M			1060 M	(testicular lesions, reduced pre- and postnatal survival, altered sexual differentiation in male offspring)	Schilling et al. 1999
130	Rat (Wistar)	90 d (F)		400			900	(tubular atrophy and degeneration)	Shaffer et al. 1945
131	Mouse (Cr1:CD-1)	126 d (F)		14[b]			140	(decreased male fertility)	Lamb et al. 1987
132	Mouse Sv/129	24 wk (F)					2400 M	(degenerative testicular lesions)	Ward et al. 1998
	Developmental								
133	Rat (Fischer- 344)	21 d ppd 1-21 1 x/d (GO)					500	(approximately 24% reduced pup body weight on ppd 21)	Cimini et al. 1994
134	Rat (Wistar)	90d preGd 90-Gd1 1 x/d (GO)		340	1700	(10% decreased fetal weight and 8% decrease in placental weight)			Nikonorow et al. 1973
135	Rat (Fischer- 344)	21d Gd 0-20 (F)		164			313	(increased prenatal and perinatal mortality)	Price et al. 1986
136	Rat (Fischer- 344)	21 d ppd 1-21 1 x/d (GO)			1000	(significant peroxisome proliferation in both liver and kidneys from pups)			Stefanini et al. 1995

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
137	Rat (Fischer- 344)	20 d Gd 0-20 1x/d (F)		357	666	(decreased fetal body weight)	1055	(fetal resorptions)	Tyl et al. 1988
138	Mouse (CD-1)	18d Gd 0-17 (F)		48			95	(increased prenatal and perinatal mortality)	Price et al. 1988c
139	Mouse (ICR)	Gd 1-18 (F)		83			170	(increased percent resorptions and dead fetuses)	Shiota et al. 1980
140	Mouse (CD-1)	17 d Gd 0-17 1x/d (F)		44			91	(external, visceral and skeletal abnormalities)	Tyl et al. 1988

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)	Reference Chemical Form
	CHRONIC EXPOSURE							
	Death							
141	Mouse (B6C3F1)	104 weeks (F)					1266 M (45% reduced survival due to hepatocellular neoplasia)	David et al. 1999, 2000b
	Systemic							
142	Rat (Sherman)	1 yr (F)	Resp	200				Carpenter et al. 1953
			Cardio	200				
			Gastro	200				
			Hemato	200				
			Hepatic	60	200	(increased liver weight at 365 days)		
			Renal	60	200	(increased kidney weight at 365 days)		
			Bd Wt	60	200	(decreased body weight gain)		
143	Rat (Sherman)	2 yr (F)	Resp	190				Carpenter et al. 1953
			Cardio	190				
			Gastro	190				
			Hemato	190				
			Hepatic	60	190	(increased liver weight)		
			Renal	190				
			Bd Wt	60	190	(decreased body weight gain for males)		
144	Rat (Fischer- 344)	2 yr (F)	Hepatic		92	(induced peroxisomal enzyme activities)		Cattley et al. 1987

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
145	Rat (Fischer- 344)	104 weeks (F)	Gastro	939 F			David et al. 1999, 2000a
			Hemato	939 F			
			Musc/skel	939 F			
			Hemato	939 F			
			Hepatic	36 F	147 M (28.2% increased liver weight and spongiosis hepatis)		
			Renal	36 F	147 M (9.8% increased relative kidney weight)		
			Endocr	939 F			
			Bd Wt		789 M (15% reduced body weight gain)		
146	Rat (Sprague-Dawley)	102 wk (F)	Hepatic		140 (morphological and enzymatic evidence of moderate peroxisome proliferation)		Ganning et al. 1991
			Bd Wt	14	140 (approximately 10% lower final body weight than controls)	1400 (approximately 27% lower final body weight than controls)	
147	Rat (Fischer- 344)	2 yr (F)	Hepatic		322 (increased incidence of foci of clear cell changes in liver)		Kluwe et al. 1982a
			Endocr		674 (anterior pituitary cell hypertrophy)		

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
148	Rat (Sprague-Dawley)	2 yr (F)	Hepatic		1377	(increased relative liver weight; increases in mitochondria, peroxisomes, lipofuscin deposits, conjugated dienes and peroxisomal enzymes)			Lake et al. 1987
149	Rat (Fischer- 344)	365 d (F)	Hepatic		947	(50% increase in relative liver weight and DNA synthesis; morphological and biochemical evidence of peroxisome proliferation)			Marsman et al. 1988
			Bd Wt		947	(final body weight reduced 17% relative to controls)			
150	Rat (Fischer- 344)	95 wk (F)	Hepatic		2444	(peroxisome proliferation, decreased catalase and increased fatty acid oxidase activity)			Rao et al. 1987
151	Rat (Fischer- 344)	108 wk (F)	Resp	2000					Rao et al. 1990
			Gastro		2000	(pseudoductular lesions in the pancreas)			
			Hepatic		2000	(100% increase in liver weight)			
			Renal		2000	(lipofuscin pigments in tubular epithelium)			
			Bd Wt				2000	(27% decrease in body weight gain)	

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
152	Rat (Wistar)	79 wk (F)	Hepatic		867 (changes in peroxisomal enzymes, increased liver weight)		Tamura et al. 1990
			Bd Wt			867 (21% decrease in body weight gain)	
153	Mouse (B6C3F1)	104 weeks (F)	Gastro	1458 F			David et al. 1999, 2000b
			Hemato	1458 F			
			Musc/skel	1458 F			
			Hepatic	117 F	292 M (30.5% increase liver weight with hepatocyte		
			Renal	117 F	354 F (increased chronic progressive nephropathy, 12% decreased relative kidney weight)		
			Endocr	1458 F			
			Bd Wt	354 F	1266 M (9.8% reduced body weight gain)		
154	Mouse (B6C3F1)	2 yr (F)	Renal	672	1325 M (chronic inflammation of the kidney)		Kluwe et al. 1982a
155	Gn Pig (NS)	1 yr (F)	Hepatic	19	64 (increase in liver weight)		Carpenter et al. 1953
			Renal	64			
			Bd Wt	64			
156	Dog (NS)	1 yr 5d/wk 1x/d (C)	Hepatic	59			Carpenter et al. 1953
			Renal	59			
			Bd Wt	59			

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
	Immunological/Lymphoreticular							
157	Rat (Sherman)	2 yr (F)		190				Carpenter et al. 1953
158	Gn Pig (NS)	1 yr (F)		64				Carpenter et al. 1953
	Reproductive							
159	Rat (Sherman)	1 yr (F)		328				Carpenter et al. 1953
160	Rat (Sherman)	2 yr (F)		190				Carpenter et al. 1953
161	Rat (Fischer- 344)	104 weeks (F)		5.8[c]		29	(bilateral testicular aspermatogenesis)	David et al. 2000a
162	Rat (Sprague-Dawley)	102 wk (F)				14	(inhibition of spermatogenesis and general tubule atrophy)	Ganning et al. 1991
163	Rat (Fischer- 344)	2 yr (F)			322	674	(severe seminiferous tubular degeneration and testicular atrophy)	Kluwe et al. 1982a
164	Rat (Wistar)	18 mo (F)				2000	(testicular atrophy)	Price et al. 1987
165	Mouse (B6C3F1)	104 weeks (F)		98.5		292	(reduced testes weights and hypospermia)	David et al. 2000b
166	Mouse (B6C3F1)	2 yr (F)		672		1325	(seminiferous tubular degeneration)	Kluwe et al. 1982a

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Cancer						
167	Rat (Fischer- 344)	2 yr (F)				1100 CEL (hepatocellular carcinoma)	Cattley et al. 1987
168	Rat (Fischer- 344)	104 wk (F)				147[d] M (CEL: 11/65 hepatocellular tumors) 939 F (CEL: 22/80 hepatocellular tumors)	David et al. 1999, 2000a
169	Rat (Fischer- 344)	78 wk (F)				1579 (CEL: 43% hepatocarcinomas, 0% in controls at week 78)	Hayashi et al. 1994
170	Rat (Fischer- 344)	2 yr (F)				322 CEL (hepatocellular carcinoma)	Kluwe et al. 1982a
171	Rat (Sprague-Dawley)	2 yr (F)				1377 CEL (hepatocellular carcinoma)	Lake et al. 1987
172	Rat (Fischer- 344)	95 wk (F)				2444 CEL (hepatocellular carcinoma)	Rao et al. 1987
173	Mouse (B6C3F1)	104 wk (F)				292[d] M (CEL: 27/65 hepatocellular tumors) 354 F (CEL: 19/65 hepatocellular tumors)	David et al. 1999, 2000b

Table 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less serious (mg/kg/day)		Serious (mg/kg/day)		
174	Mouse (B6C3F1)	2 yr (F)					672	CEL (hepatocellular carcinoma)	Kluwe et al. 1982a

[a]The number corresponds to entries in Figure 3-2.

[b]Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.1 mg/kg/day; The MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

[c]Used to derive a chronic-duration oral minimal risk level (MRL) of 0.06 mg/kg/day. The MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

[d]Differences in levels of health effects and cancer effects between males and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); DNA = deoxyribonucleic acid; (F) = feed; F = female; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day; Gn pig = guinea pig; (GO) = gavage oil; Hemato = hematological; LOAEL = lowest-observed-effect level; Ld = lactation day; LD_{50} = lethal dose, 50% kill; mo = month(s); M = male; NOAEL = no-observed=adverse=effect level; NS = not specified; ppd = postpartum day(s); Resp = respiratory; wk = week(s); x = time(s); yr = year(s)

Figure 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral

Acute (≤14 days)

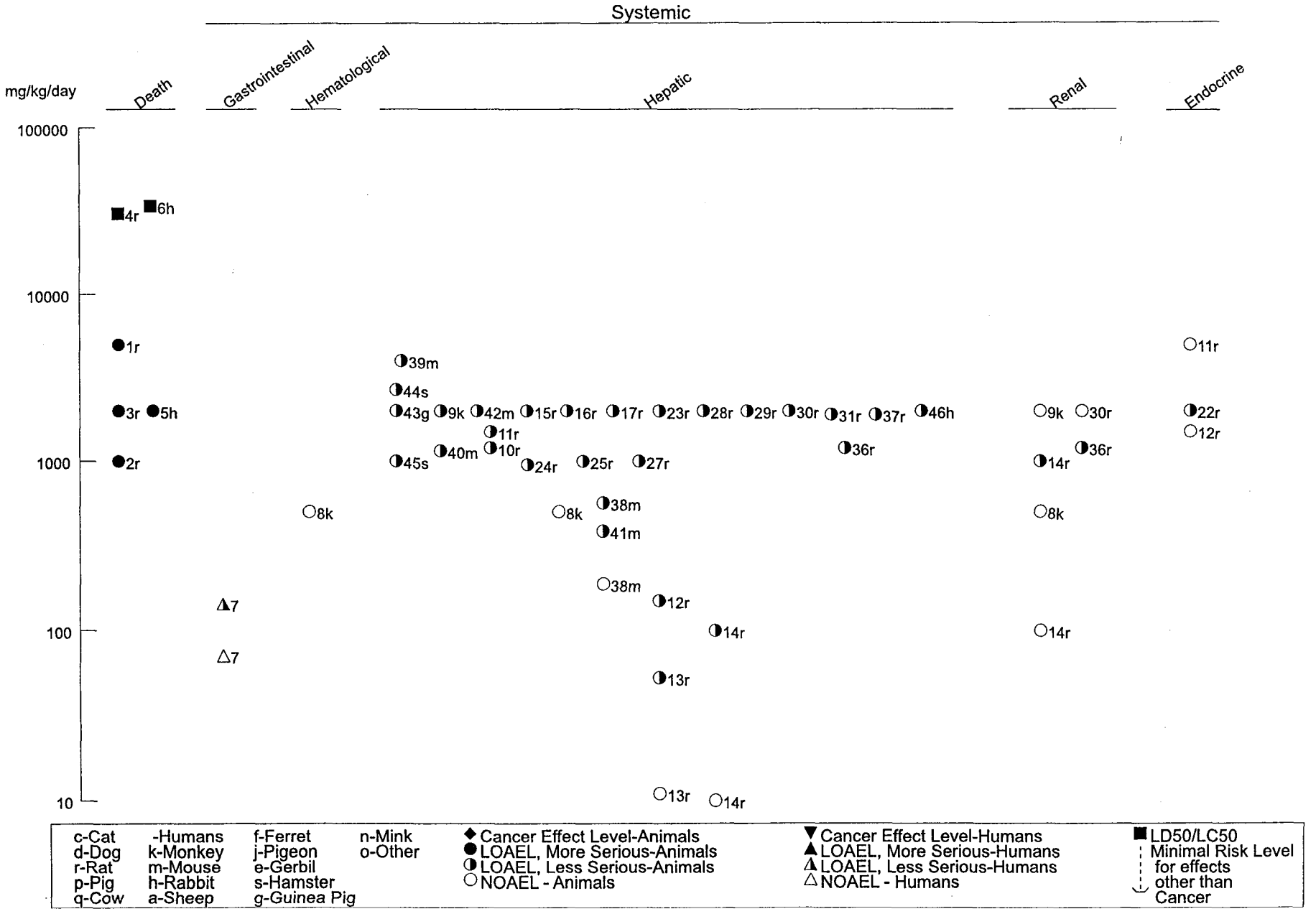


Figure 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (*Continued*)

Acute (≤14 days)

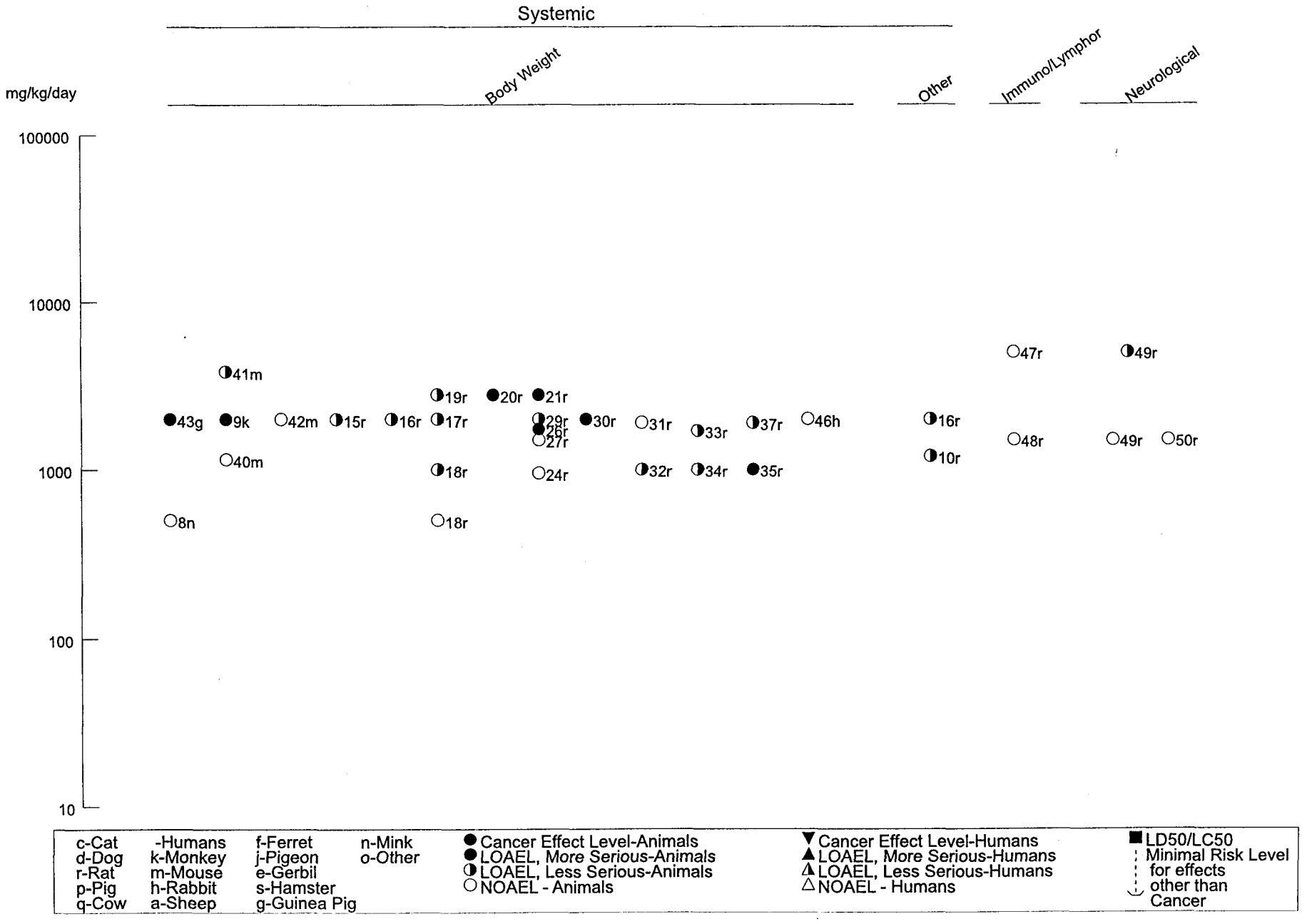


Figure 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (*Continued*)

Acute (≤14 days)

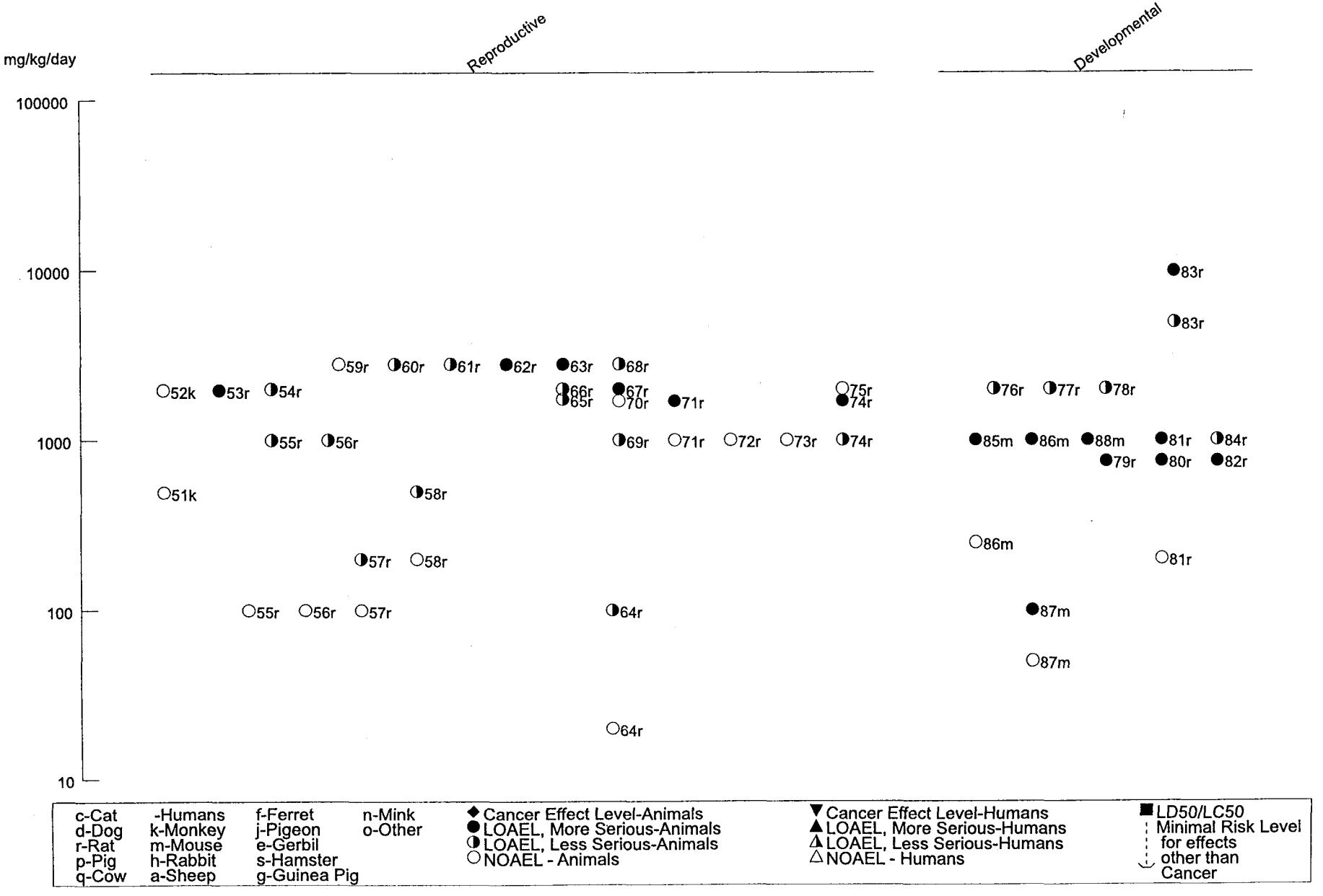


Figure 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (*Continued*)

Intermediate (15-364 days)

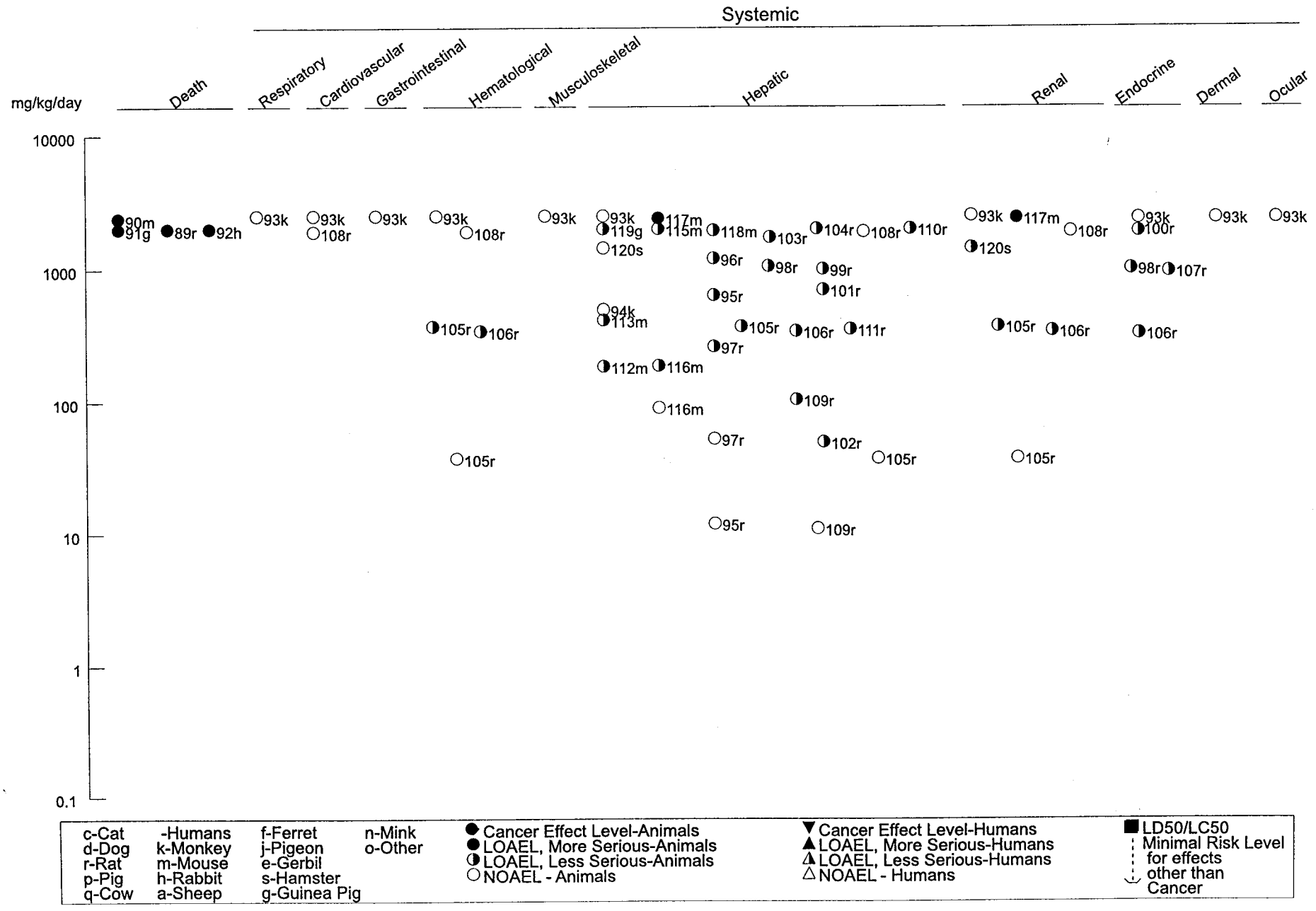


Figure 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (*Continued*)

Intermediate (15-364 days)

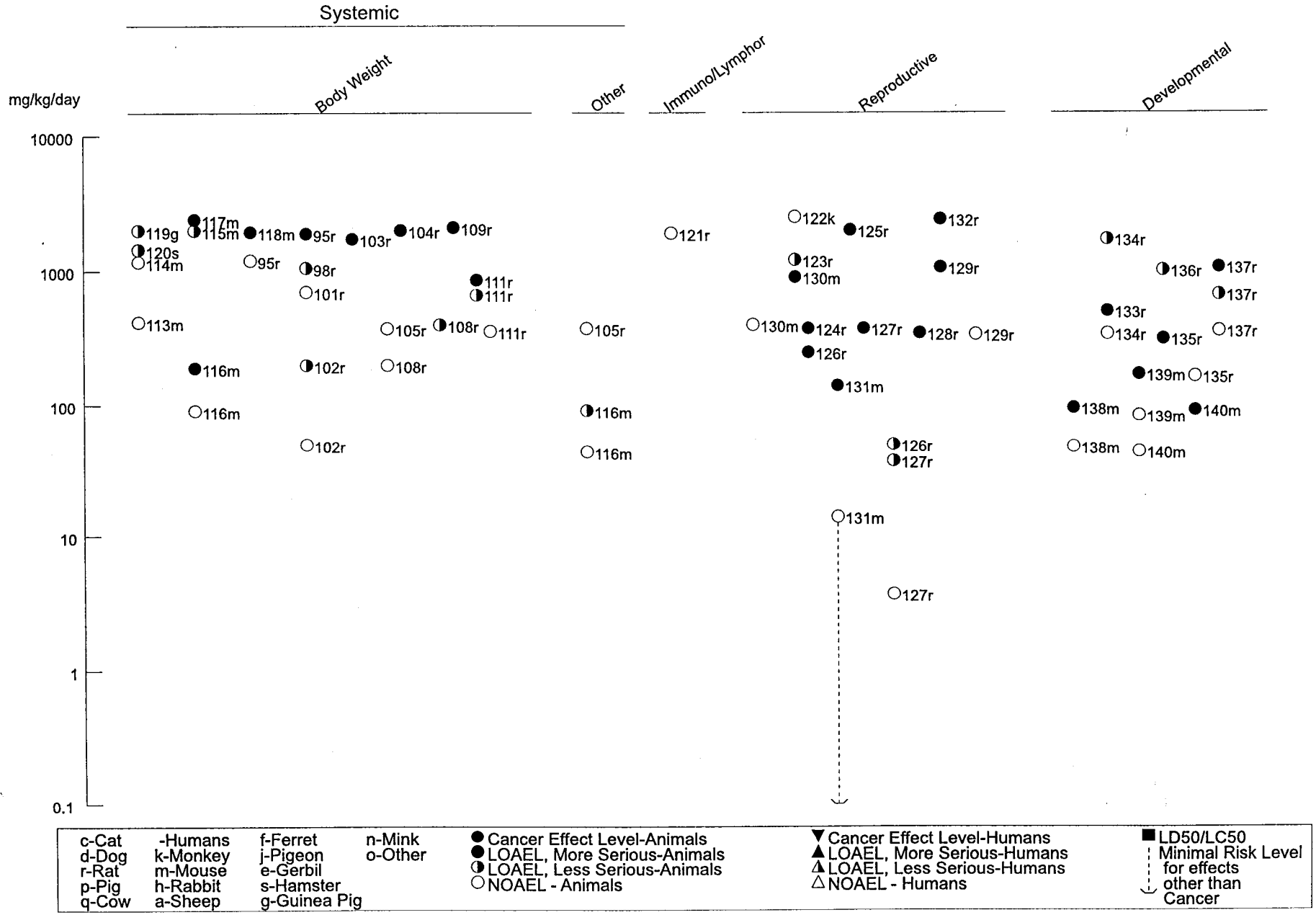


Figure 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (*Continued*)

Chronic (≥365 days)

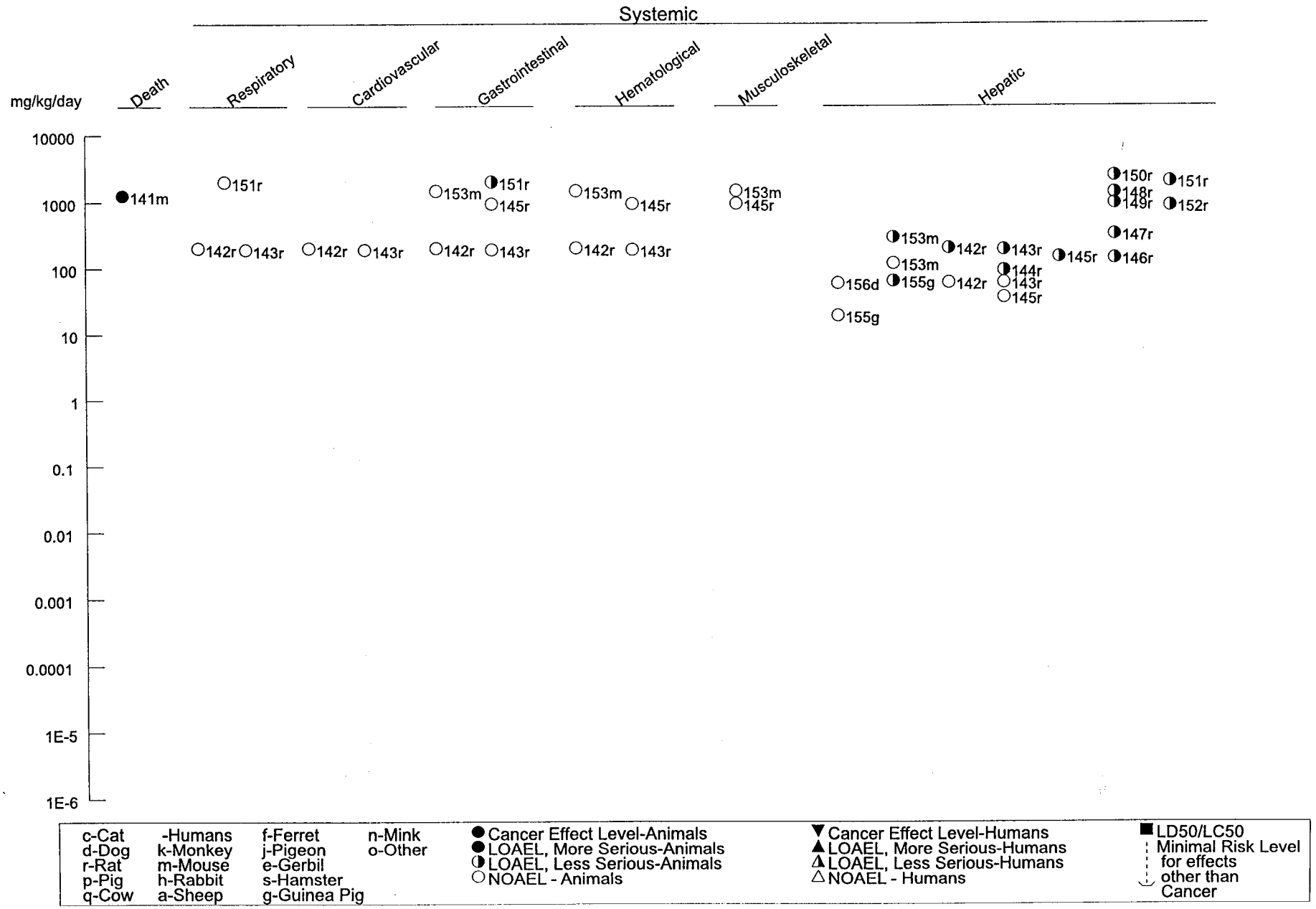
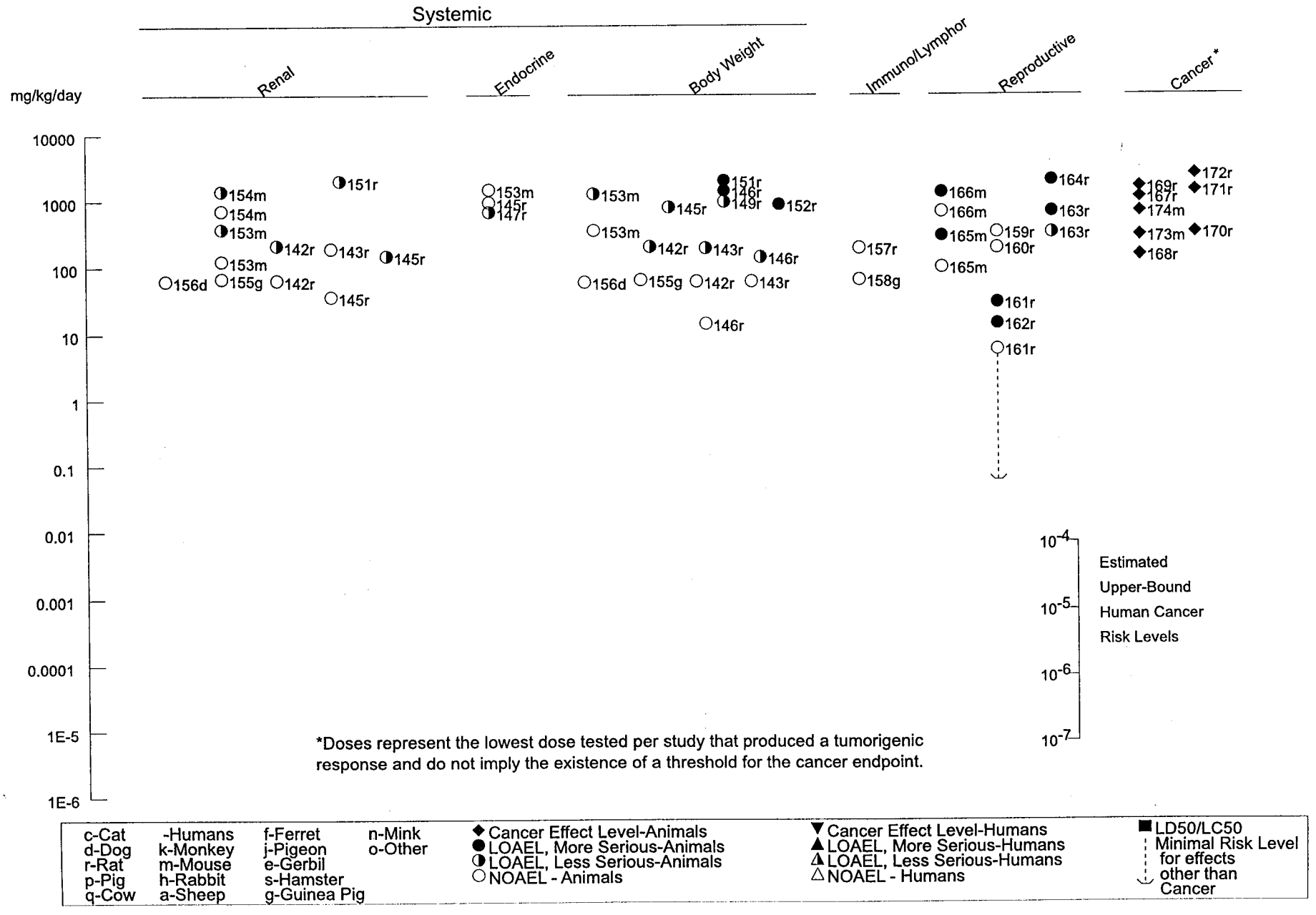


Figure 3-2. Levels of Significant Exposure to Di-(2-ethylhexyl)phthalate - Oral (*Continued*)

Chronic (≥365 days)



Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to DEHP. No adverse respiratory effects were reported in any of the animal studies reviewed. However, no study was located that evaluated pulmonary function.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to DEHP. No adverse cardiovascular effects were reported in any of the animals studies reviewed. However, no study was located that evaluated cardiovascular function in animals following oral exposure.

A potential effect in human heart muscle contractility was identified in *in vitro* studies. Mono(2-ethylhexyl)phthalate (MEHP) (a product of DEHP hydrolysis) displayed a dose-dependent negative inotropic effect on human atrial trabecula (Barry et al. 1989, 1990). This suggests the possibility that high levels of serum MEHP could have a cardiotoxic effect in humans. However, rapid metabolism of MEHP would act to minimize the probability that MEHP concentrations would reach the concentration associated with the negative inotropic effect. The authors suggested that infants with multisystem failures would be the group at greatest risk to a cardiotoxic effect of MEHP. In contrast to the *in vitro* studies, there was no indication of cardiovascular effects in 18 infants who had increased plasma levels of DEHP (8.3±5.7 Fg/mL, mean highest concentration) from exposure during extracorporeal membrane oxygenation (ECMO) therapy (DEHP had leached from plastic tubing) (Karle et al. 1997). Cardiac performance was evaluated by using echocardiograms to estimate output from heart rate, systolic blood pressure, left ventricular shortening fraction, and stroke volume measurements.

Gastrointestinal Effects. Acute exposures to large oral doses of DEHP can cause gastrointestinal distress. When two humans were given a single oral dose of 5 or 10 g DEHP, the individual consuming the larger dose complained of mild abdominal pain and diarrhea (Shaffer et al. 1945). No other effects of exposure were noted. No adverse gastrointestinal effects were reported in any of the animal studies reviewed with the exception of pseudoductular lesions in the pancreas of rats administered 3,000 mg DEHP/kg/day in the diet for 108 weeks (Rao et al. 1990). The significance of this finding is unclear because pancreatic lesions were not observed in rats and mice that were similarly exposed to dietary DEHP at doses #939 and 1,458 mg/kg/day, respectively, for 104 weeks (David et al. 2000a, 2000b).

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to DEHP. There were no hematological changes in male Cynomolgus monkeys that were administered 500 mg DEHP/kg/day by gavage for 14 consecutive days (Pugh et al. 2000). Exposure of male albino rats to doses of 200–1,900 mg/kg/day DEHP in the diet for 90 days had no effect upon red blood cell counts, hemoglobin levels, or differential white cell counts (Shaffer et al. 1945). In contrast, a recent 13-week dietary study in Sprague-Dawley rats reported slight but significant decreases in red blood cell counts and serum hemoglobin in males that received approximately 375.2 mg DEHP/kg/day (Poon et al. 1997); a dose of 37.6 mg DEHP/kg/day was without effect. In a separate experimental series in the same study, it was reported that doses of 345 and 411 mg DEHP/kg/day (only levels tested) in male and female rats, respectively, significantly increased blood cell counts and decreased mean corpuscular hemoglobin in females, and significantly increased platelet counts in both males and females (Poon et al. 1997). There is no apparent explanation for the inconsistent results between the Shaffer et al. (1945) and Poon et al. (1997) studies other than the fact that the Poon et al. (1997) values might still have been within the normal range for the rats; no statistical analysis was presented in the Shaffer et al. (1945) study. No adverse hematological effects were reported in an intermediate-duration gavage study in marmoset monkeys administered up to 2,500 mg DEHP/kg/day (Kurata et al. 1998). Exposures of rats to 200 mg/kg/day for 1 year caused no changes in erythrocyte or total and differential leukocyte counts (Carpenter et al. 1953). Similarly, comprehensive hematological evaluations showed no toxicologically significant changes in F344 rats and B6C3F1 mice that were fed DEHP in the diet at doses #939 or 1,458 mg DEHP/kg/day, respectively, for 104 weeks (David et al. 2000a, 2000b).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans and no reports of musculo/skeletal effects in animals were found in any of the studies reviewed.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to DEHP. Limited information on hepatic effects in humans exposed to DEHP is available from studies of dialysis patients and cultured human hepatocytes. In one individual there was an increased number of liver peroxisomes after 1 year, but not after 1 month of treatment (Ganning et al. 1984, 1987). A serious limitation of this observation is that repeat biopsies were not obtained from the same patient, so that an appropriately controlled analysis is not possible. Additionally, analysis of liver biopsies from patients receiving other kinds of hypolipidemic drugs has not yielded any evidence for peroxisomal proliferation (Doull et al. 1999). Recognizing some limitations of using primary hepatocytes *in vitro* because of their tendency to lose some metabolic capabilities (Reid 1990), in cultured human hepatocytes there were no changes in the activities of peroxisomal palmitoyl-CoA oxidase and/or carnitine acetyltransferase when

the cells were exposed to MEHP (Butterworth et al. 1989; Elcombe and Mitchell 1986; Hasmall et al. 2000).

There are abundant animal data detailing the effects of DEHP on liver structure and function. Rats and mice are most susceptible to the hepatic effects of DEHP, while dogs and monkeys are less likely to experience changes in the liver after exposure. In general, the data indicate that male rats are more susceptible to the hepatic effects of DEHP than are females.

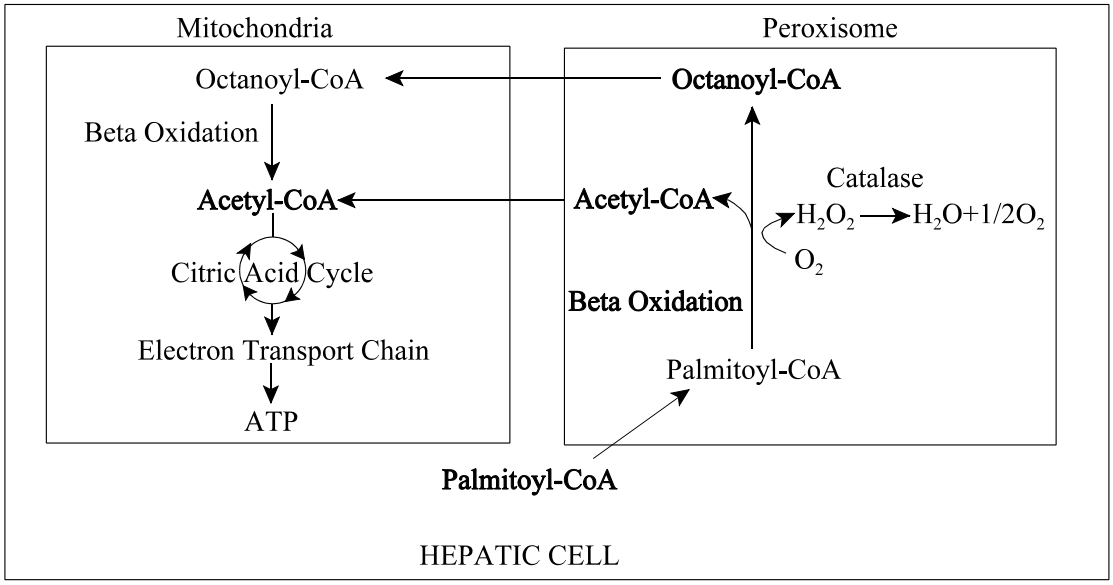
Hyperplasia/Hypertrophy. Oral exposures of rats and mice to DEHP characteristically result in a marked increase in liver mass (Barber et al. 1987; Berman et al. 1995; Carpenter et al. 1953; David et al. 1999, 2000a, 2000b; DeAngelo et al. 1986; Dostal et al. 1987a, 1987b; James et al. 1998; Lake et al. 1986; Lamb et al. 1987; Marsman et al. 1988; Mitchell et al. 1985b; Oishi 1989a, 1994; Parmar et al. 1988; Poon et al. 1997; Rao et al. 1990; Rhodes et al. 1986; Shin et al. 1999; Takagi et al. 1990; Tamura et al. 1990; Tomaszewski et al. 1988; Tyl et al. 1988). This is due to rapid cell division (hyperplasia), along with some enlargement of cells (hypertrophy). A 14-day exposure of rats to 1,200 mg/kg/day increased the relative liver weight by 52% after 1 week and 74% after 2 weeks (Takagi et al. 1990). Relative liver weight was significantly increased in F344 rats (28.2% higher than controls) and B6C3F1 mice (30.5% higher than controls) that were fed DEHP in the diet at doses of $147 and $292 mg/kg/day, respectively, for 104 weeks (David et al. 2000a, 2000b).

Hepatic hyperplasia appears to be the initial physiological response to DEHP exposure in rats (Busser and Lutz 1987; Smith-Oliver and Butterworth 1987). When rats were exposed to single doses of $150 mg DEHP/kg, there was an increase in cell division within 24 hours (Berman et al. 1995; Busser and Lutz 1987; Smith-Oliver and Butterworth 1987). Treatment for 2 days with approximately 1,000 mg DEHP/kg significantly increased relative liver weight and DNA synthesis in rats and to a lesser extent in mice (James et al. 1998). During the early stages of a chronic study, repeated oral doses of $50 mg/kg/day increased mitotic activity when given to rats for 3 consecutive days (Mitchell et al. 1985b). The increase in mitosis occurred only in the early stages of treatment and did not persist beyond the first week of exposure in studies with 3–12-month durations (Marsman et al. 1988; Mitchell et al. 1985b; Smith-Oliver and Butterworth 1987). The observation that DEHP causes an early transient increase in liver DNA synthesis above a certain dose level is similar to phenobarbital, a known rodent liver tumor promoter (Dalton et al. 2000), and strengthens the conclusion that DEHP is an epigenetic tumor promoting agent in rodents as discussed in Section 3.5.2 (Mechanisms of Toxicity).

Morphology. Morphological changes which were observed in the livers of treated rats included fat deposits in the periportal area, a decline in centrilobular glycogen deposits, and structural changes in the bile ducts (Mitchell et al. 1985b; Price et al. 1987). Liver cells became enlarged and lipofuscin deposits accumulated, indicating that peroxidation of cellular lipids had occurred (Lake et al. 1987; Mitchell et al. 1985b; Price et al. 1987). On a microscopic level, there was a definite increase in hepatic peroxisomes in the centrilobular and periportal areas of the liver and there was often an increase in the number of mitochondria (Hodgson 1987; Nair and Kurup 1987a). Lipid filled lysosomes were observed in some cases (Mitchell et al. 1985b). Each of these changes contributed to cellular hypertrophy. Many of the morphological changes described above were seen in the male rats at doses $50 mg/kg/day but did not appear in the females until doses of 200 mg/kg/day and above (Mitchell et al. 1985b), indicating that male rats are somewhat more susceptible than are females. Histopathological changes in the liver of rats that were exposed to DEHP in the diet for up to 104 weeks included spongiosis hepatis at doses $147 mg/kg/day, and diffuse hepatocellular enlargement, increased cytoplasmic eosinophilia, and increased Kupffer cell or hepatocyte pigmentation at $789 mg/kg/day (David et al. 2000a). Hepatic effects in mice that were similarly exposed to DEHP for 104 weeks included hepatocellular enlargement and increased hepatocyte pigmentation, cytoplasmic eosinophilia, and chronic inflammation at doses $1,266 mg/kg/day (David et al. 2000b).

Peroxisome Proliferation. An increase in hepatic peroxisomes and induction of peroxisomal enzymes are markers of DEHP exposure in rodents. Acute exposures to doses of 1,000 mg/kg/day or greater were consistently associated with an increase in cellular peroxisomes (Ganning et al. 1989; Rhodes et al. 1986; Shin et al. 1999). Along with the increase in the number of peroxisomes, there is a simultaneous increase in the activities of many of the peroxisomal enzymes, especially those related to the catabolism of fatty acids. Peroxisomes are organelles that utilize molecular oxygen and produce hydrogen peroxide during substrate catabolism (Figure 3-3). They contain a variety of enzymes including oxidases, peroxidases, and catalase (McGilvery and Goldstein 1983). Peroxisomal fatty acid oxidation follows the same pathway utilized by mitochondria except that ATP is not generated and hydrogen peroxide is produced in place of water. Subsequently catalase and peroxidases reduce hydrogen peroxide to water. Peroxisomal fatty acid oxidation cannot completely degrade a fatty acid to acetyl-coenzyme A (CoA). Octanoyl CoA is exported to the mitochondria for the completion of oxidation by way of the citric acid cycle and

Figure 3-3. Peroxisomal Fatty Acid Metabolism



Adapted from Stott 1988

electron transport chain (Figure 3-3). Accordingly, proliferation of peroxisomes and increased fatty acid catabolism by this organelle requires a simultaneous increase in mitochondrial fatty acid metabolism.

There are multiple changes in peroxisomal enzymes that occur following exposures to DEHP. Some of the changes are due to peroxisomal proliferation, but induction of specific enzymes is also apparent. There is considerable agreement among studies that indicate that the activities of the enzymes responsible for fatty acid catabolism (palmitoyl-CoA oxidase, enoyl-CoA hydratase, carnitine acyltransferase, and α-glycerophosphate dehydrogenase) were increased in rodents after exposure to DEHP by factors as great as 1,500% (Cattley et al. 1988; David et al. 1999; Dostal et al. 1987a; Elliott and Elcombe 1987; Ganning et al. 1991; Lake et al. 1986; Poon et al. 1997; Rhodes et al. 1986; Ward et al. 1998). Some evidence of peroxisomal enzyme induction was apparent in rats within 3 weeks at a dose of 50 mg/kg/day (Barber et al. 1987; Mitchell et al. 1985b; Short et al. 1987) but not with doses as low as 5 and 11 mg/kg/day (Barber et al. 1987; Short et al. 1987). Chronic administration of 14 mg/kg/day DEHP to rats caused a gradual increase in the activities of selected enzymes (palmitoyl Co-A oxidase, carnitine acetyltransferase) over a period of 102 weeks. At the end of this time, enzyme activities were more than twice the values for the controls (Ganning et al. 1991).

If fatty acid catabolism by peroxisomes is increased by DEHP exposure and excess hydrogen peroxide is produced, there is an increased requirement for detoxification by peroxisomal catalase and the cellular peroxidases. However, the data concerning the impact of DEHP on catalase in rats are inconsistent. There are reported dose and duration conditions where the activity of catalase decreased (Ganning et al. 1989; Rao et al. 1987), where it remained constant (Elliott and Elcombe 1987; Perera et al. 1986), and where it increased (Perera et al. 1986; Tamura et al. 1990). Both increases and decreases in catalase were reported following various durations of exposure of rats to doses of 14, 140, or 1,400 mg/kg/day DEHP over a 102-week period (Ganning et al. 1991). In time course studies of 79 weeks at 867 mg/kg/day (Tamura et al. 1991) and 52 weeks at 6,000 mg/kg/day (Conway et al. 1989), increases in rat liver catalase were sustained. It should be noted that increases in catalase in these studies did not exceed 200% of control levels.

In addition to catalase, the enzymes glutathione peroxidase and superoxide dismutase are important elements in the cellular defenses against free radical oxygen. Cytoplasmic activity of glutathione peroxidase was decreased in rats by 14 and 28 days of exposure to doses of 500–2,000 mg/kg/day DEHP (Elliott and Elcombe 1987; Perera et al. 1986) and superoxide dismutase activity was decreased by a 28 day exposure of rats to 1,000 and 2,000 mg/kg/day (Elliott and Elcombe 1987). Glutathione

peroxidase activity in rats was also found to be depressed to about 50% of control values throughout 79 weeks of exposure to 867 mg/kg/day DEHP (Tamura et al. 1990) and throughout 52 weeks of exposure to 600 mg/kg/day (Conway et al. 1989).

Peroxidases and catalase are present in hepatic cells and are able to detoxify the peroxide produced by the peroxisomes. Fluctuations in levels of glutathione peroxidase and catalase activity following DEHP treatment in rats might indicate that the capacity to oppose an increased production of hydrogen peroxide by the peroxisomes is limited. It can also be postulated that a variety of exogenous factors such as diet, and other metabolic demands on the liver might govern the capacity of the hepatic free radical defense system to respond to the increased peroxisomal production of hydrogen peroxide. In addition to the enzymes mentioned above, the lipid ubiquinone, which acts as an antioxidant protecting against lipid oxidation and also protein and DNA oxidation, was significantly increased after treatment with approximately 2,500 mg DEHP/kg/day for 21 days in rats of various ages (Turunen and Dallner 1998). The authors suggested that this increase might be a response to oxidative stress. DEHP (1,000 mg/kg/day) given in the diet of rats for 30 days also increased cellular ubiquinone, by 250% (Nair and Kurup 1987b).

When peroxisomal catabolism of fatty acids is not accompanied by an increase in the ability of the liver to detoxify hydrogen peroxide, the excess hydrogen peroxide might react with cellular lipids, proteins, and nucleic acids (Reddy et al. 1986). Slight but significant increases in malondialdehyde and conjugated dienes (markers for the reaction of peroxides with fatty acids) were seen in rat hepatic cells following 28 days of exposure to 2,000 mg/kg/day DEHP (Elliott and Elcombe 1987). The catalase activity was unchanged while glutathione peroxidase activity was decreased. In a separate study, there was no increase in oxidized lipids in exposed livers as indicated by malondialdehyde concentrations following 79 weeks of dietary exposure to 867 mg/kg/day DEHP (Tamura et al. 1990). Catalase activity was increased and glutathione peroxidase activity decreased. Higher dose levels were not tested. Lipofuscin deposits, a long-term marker for lipid reactions with peroxides, were identified in the livers of rats exposed to between 500 and 2,000 mg/kg/day DEHP for their lifetime (Cattley et al. 1987; Price et al. 1987). Thus, there are some data to support the hypothesis that at least a portion of the hepatic damage induced by DEHP is the result of the reaction of hydrogen peroxide with cellular lipids.

Serum and Tissue Lipids. A decrease in circulating cholesterol and triglyceride levels is also associated with DEHP exposure in rats (Bell 1982; Dostal et al. 1987a; Eagon et al. 1994; Mocchiutti and Bernal 1997; Oishi 1989a; Poon et al. 1997; Rhodes et al. 1986), but not in primates (Rhodes et al. 1986).

Increased fatty acid catabolism decreases the concentration of free fatty acids available for export from the liver as circulating triglycerides. This provides a rationale for the lowered triglyceride values. The lowered serum cholesterol concentration apparently results from inhibition of cholesterol synthesis and stimulation of the conversion of cholesterol to bile acids in the liver (Nair and Kurup 1986).

Studies by Bell and coworkers have shown that DEHP also can alter sterologenesis in rodents, which may have an impact on steroid-dependent functions, such as reproductive functions. For example, feeding female rats DEHP at an estimated dose of 500 mg/kg/day for 13 days significantly inhibited sterologenesis from ^{14}C-mevalonate in liver and adrenal minces (Bell 1980). DEHP also inhibited cholesterol synthesis in the liver from male rats and rabbits as well as in rats' testes (Bell 1982). In a subsequent study, Bell and Buthala (1983) demonstrated that the inhibition of cholesterol synthesis in the liver was due to a reduction in the activity of microsomal acylCoA:cholesterol acyltransferase, an enzyme responsible for the esterification of cholesterol.

Mixed Function Oxidase Enzymes. The mixed function oxidase (MFO) system is a second hepatic enzyme system which appears to be affected by DEHP in rodents (Ganning et al. 1991; Hodgson 1987; James et al. 1998; Parmar et al. 1988; Rhodes et al. 1986; Short et al. 1987), but not in monkeys (Kurata et al. 1998; Rhodes et al. 1986). The MFO system consists of cytochrome P-450, a series of hydratases and hydroxylases. Peroxisome proliferators particularly induce P-450 isoenzymes of the CYP4A subfamily, which have fatty acid omega and omega-1 hydroxylase activity. Significant induction of fatty acid omega hydoxylase and P-450 4A1 mRNA were reported following DEHP administration to rats (Sharma et al. 1988, 1989). Changes in hepatic levels of cytochrome P-450, NADPH Cytochrome c reductase, lauryl-11- and 12-hydroxylase, ethoxycoumarin-O-deethylase, ethylmorphine-N-demethylase, and aniline hydroxylase were increased by DEHP exposure in rats by doses as low as 50 mg/kg/day (Ganning et al. 1991; Hodgson 1987; Mitchell et al. 1985b; Parmar et al. 1988; Rhodes et al. 1986; Short et al. 1987). A comparative study in rats, hamsters, and mice, using immunochemical techniques, showed that mice were the most responsive species for induction of hepatic microsomal carboxylesterases after 7 days of dosing with DEHP, whereas rats were less responsive and hamsters were poorly responsive or unresponsive, particularly in butinilicaine hydrolase activity (Hosokawa et al. 1994). Age-dependent effects on enzyme activities were examined in rats of three ages, 3, 6, and 10 weeks old (Parmar et al. 1994). Single administration of 2,000 mg DEHP/kg decreased the cytochrome P-450 contents in the liver and activity of aryl hydrocarbon hydroxylase (AHH), aniline hydroxylase, and ethylmorphine N-demethylase in all age groups, while repeated exposure induced them with maximum increases occurring in 3-week-old rats. Administration of DEHP for 15 days decreased cytochrome

P-450 and the activity of the three enzymes only in the 3-week-old rats. Six- and 10-week-old rats showed an inhibition of AHH and increased activity of aniline hydroxylase and ethylmorphine N-demethylase, which were lower than that seen after 7 days of exposure in their respective groups. The effect of the changes in the MFO enzymes on the liver is difficult to evaluate. Although the MFO system tends to process various foreign chemicals and thus be of benefit, some of the oxidized intermediary metabolites produced by the initial MFO reactions are more toxic than are the parent compounds.

Carbohydrate Metabolism. Some impact of DEHP on carbohydrate metabolism in the liver of rats has also been observed. Glycogen deposits decline with DEHP exposure. This might be the result of a need to mobilize glucose for use in the liver. Since exposure to as little as 50 mg/kg/day DEHP for 28 days decreases the activity of hepatic glucose-6-phosphatase in rats (Mitchell et al. 1985b), the transport of glucose from the liver is limited. Therefore, the decline in hepatic glycogen might be the result of increased hepatic glucose utilization. Some of the glucose could be metabolized to produce the reducing equivalents necessary for the activity of glutathione peroxidase. This suggestion is supported by the finding that glucose-6-phosphate dehydrogenase activity is increased in both male and female rats exposed to 50–2,000 mg/kg/day DEHP (Gerbracht et al. 1990). The activities of glyceraldehyde-3-phosphate dehydrogenase, malic enzyme (extramitochondrial), and lactic dehydrogenase were also increased (Gerbracht et al. 1990). These enzymes are used during hepatic catabolism of glucose and produce intermediary metabolites that are used, among other things, for the manufacturing of new cells during hepatic hyperplasia.

Membrane Structure. The effect of DEHP on liver metabolism might be modulated through a change in the structure of the cell membranes. Both membrane proteins and lipids are altered with DEHP exposure (Bartles et al. 1990; Edlund et al. 1987; Ganning et al. 1987; Gupta et al. 1988). Following 15 days of dietary exposure to 1,000 mg/kg/day DEHP, the concentration of membrane protein CE-9 was increased in rats. This protein appears to be related to transport of the biochemical signal which stimulates peroxisome proliferation. Other membrane protein concentrations were decreased with DEHP exposure in rats including epidermal growth factor receptor, asialoglycoprotein receptor, dipeptidylpeptidase-IV, HA-312, and HA-4 (Bartles et al. 1990; Gupta et al. 1988). There was an increase in the concentrations of the membrane lipids dolichol and dolichol phosphate upon the introduction of DEHP into the diet of rats (Edlund et al. 1987; Ganning et al. 1987). Dolichol phosphate participates in the synthesis of membrane glycoproteins. Accordingly, glycoprotein membrane receptor sites could be affected by DEHP through this mechanism leading to altered movement of materials across membranes and signaling changes in cell metabolism. A recent study reported that administration of 1,200 mg DEHP/kg/day for

3 days to rats resulted in significant increases in total liver lipid content and total phospholipid (Adinehzadeh and Reo 1998). Moreover, DEHP significantly increased the phosphatidylcholine, phosphatidylethanolamine, and cardiolipid in the liver.

Other Species. The discussion of hepatic effects in the above subsections is based on effects in rats and mice. Not all animal species are equally susceptible to the hepatic effects of DEHP. Differences in responsiveness are particularly evident with respect to the increases in peroxisomal content of liver cells, induction of peroxisomal enzymes, and increased liver weight (hypertrophy and hyperplasia). Although these responses clearly occur in rats and mice, hamsters are only partially responsive and guinea pigs, and monkeys are refractory.

Treatment of male rats for 14 days with 100 mg/kg/day of DEHP resulted in a 20% increase in relative liver weight, while in the same study, treatment of male hamsters with a 10-fold higher dose, 1,000 mg/kg/day, also resulted in a 20% increase in relative liver weight, and 250 mg/kg/day DEHP had no effect (Lake et al. 1984a). In a study comparing male rats and guinea pigs, 950 mg/kg/day DEHP for 4 days resulted in increased liver weight, peroxisomal fatty acyl CoA oxidase activity, peroxisome volume fraction, and hyperplasia in rats but not guinea pigs (Hasmall et al. 2000). In a study of male and female marmoset monkeys, 500 or 2,500 mg/kg/day DEHP for 13 weeks had no effect on relative liver weight, peroxisome volume density, or peroxisomal fatty acyl CoA oxidase activity (Kurata et al. 1998). In another marmoset study, 200 mg/kg/day DEHP for 14 days had no effect on relative liver weight, peroxisome area density, or peroxisomal fatty acyl CoA oxidase activity (Rhodes et al. 1986). Male cynomologus monkeys treated with 100 or 500 mg/kg/day DEHP by gavage for 25 days had no changes in relative liver weight or peroxisomal fatty acyl CoA oxidase activity (Short et al. 1987). Similarly, there were no effects on liver weight, liver histology, or hepatic markers for peroxisomal proliferation, including replicative DNA synthesis, peroxisomal beta-oxidation activity, and gap junctional intercellular communication, in male Cynomolgus monkeys that were administered 500 mg DEHP/kg/day by gavage for 14 consecutive days (Pugh et al. 2000). Since the reversible inhibition of gap junctional intercellular communication has been hypothesized to contribute to the mechanism of tumor promotion, and since most, if not all, tumor promoters, including DEHP (Malcolm and Mills 1983, 1989), have been shown to block gap junctional communication both *in vitro* and *in vivo* (Sai et al. 2000; Trosko 2001; Trosko and Chang 1988; Trosko et al. 1995), the lack of DEHP's ability to inhibit gap junctional communication in the monkey where no tumor promotion occurs would be consistent with the results obtained.

Dogs also appear to be resistant to hepatic effects from DEHP exposure. In dogs given doses of up to 59 mg/kg/day DEHP for 1 year, there were no observed changes in liver weight or structure. Liver function as measured by sulfobromophthalein retention was not affected (Carpenter et al. 1953). Rabbits, on the other hand, experienced a significant decrease in relative liver weight following 7 days of exposure to 2,000 mg/kg/day DEHP (Parmar et al. 1988). There was a corresponding decrease in the activities of the MFO liver enzymes. Continued administration of this dose was lethal to the rabbits within 14 days.

The role of peroxisome proliferation in DEHP-induced hepatocarcinogenesis in rodents and related issues are discussed in Section 3.5.2.

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to DEHP. A possible role of DEHP in polycystic kidney disease in long-term hemodialysis patients has been suggested (Bommer et al. 1985; Crocker et al. 1988; Krempien and Ritz 1980), but there is no conclusive evidence for such an association. No association can be inferred because significant confounding variables related to the compromised health status of dialysis patients usually preclude attributing a key role to DEHP.

Studies in animals have provided an inconsistent picture. For example, acute (5–14-day) exposures of rats to doses of 1,000–1,200 mg/kg/day resulted in moderate increases in relative kidney weights (Dostal et al. 1987a; Takagi et al. 1990). In the Dostal et al. (1987a) study, the increase was observed in 21-, 42- and 86-day-old rats, but not in younger rats. In other studies, gavage administration of 1,200 mg DEHP/kg/day for 3 days induced a 2–3-fold increase in kidney microsomal lauric acid omega-hydroxylation activity in rats (Sharma et al. 1989), although gavage doses of 2,000 mg/kg/day administered to rats or marmoset monkeys for 14 days did not significantly alter kidney weights (Rhodes et al. 1986). There were no changes in kidney weight or histology in Cynomolgus monkeys that were exposed to 500 mg DEHP/kg/day by gavage for 14 consecutive days (Pugh et al. 2000).

In intermediate-duration studies, doses of 1,900 mg/kg/day administered to rats for 90 days did not significantly alter kidney weights (Shaffer et al. 1945), but doses of approximately 375 mg/kg/day for 13 weeks significantly increased relative kidney weight in a more recent rat study (Poon et al. 1997). A 24-week feeding study of 2,400 mg/kg/day DEHP reported degenerative renal lesions in Sv/129 wild type (+/+) mice prior to death, but only minimal renal lesions in knockout (-/-) type mice for PPARα (Ward et al. 1998). Hamsters fed approximately 1,436 mg DEHP/kg/day for 30 weeks showed a significant increase in relative kidney weight, but no histopathological alterations (Maruyama et al. 1994). No

adverse renal effects were reported in marmoset monkeys treated daily by gavage for 13 weeks with up to 2,500 mg DEHP/kg/day (Kurata et al. 1998).

In chronic-duration studies, effects observed in rats that were exposed to DEHP in the diet for 104 weeks included an increased incidence and severity of mineralization of the renal papilla in males at doses $5.8 mg/kg/day, increased relative kidney weight in both sexes at $147 mg/kg/day, and increased severity of normally occurring renal tubule pigmentation and chronic progressive nephropathy in both sexes at $789 mg/kg/day (David et al. 2000a). There were no significant changes in urine volume, urine creatinine concentration, creatinine clearance, or other urinalysis parameters in this study. Dietary administration of DEHP similarly caused increased kidney weights in rats that were exposed to 200 mg/kg/day for 1 year, although no significant increase was observed after 2 years (Carpenter et al. 1953), and lipofuscin pigments in the tubular epithelium of rats exposed to 3,000 mg DEHP/kg/day for 108 weeks (Rao et al. 1990). Gavage administration of an average dosage of 0.92 mg DEHP/kg/day for 1 year caused focal inflammatory changes accompanied by cystic dilation of the tubules in three of eight rats, as well as diminished mean creatine clearance in seven rats (Crocker et al. 1988). The lesions were consistent with spontaneous nephropathy commonly observed in old rats and suggested that treatment with DEHP might accelerate the onset of the lesion in younger rats. The significance of these findings is unclear due to the small number of animals, treatment of some animals with a leachate from an artificial kidney unit, unreported rat strain, and bolus method of exposure. Renal effects observed in mice that were exposed to DEHP in the diet for 104 weeks included increased severity of naturally occurring chronic progressive nephropathy at $354 mg/kg/day, but no toxicologically significant changes in urinalysis indices (David et al. 2000b). An increased incidence of chronic inflammation of the kidney was similarly observed in mice that ingested approximately 1,325 mg DEHP/kg/day in the diet for 2 years (Kluwe et al. 1982a). No adverse renal effects were seen in guinea pigs exposed to 64 mg DEHP/kg/day for 1 year, or in dogs exposed to 59 mg/kg/day for 2 years (Carpenter et al. 1953; Rhodes et al. 1986). The relevance of the kidney effects observed in the dietary studies in rats and mice is unclear because some of the findings (Crocker et al. 1988; David et al. 2000a, 2000b) indicate that they likely reflect exacerbation of age-, species-, and/or sex-related lesions by DEHP and are not accompanied by changes in kidney function. The mechanism of the effect induced by the lowest dietary dose of DEHP (i.e., mineralization of the renal papilla in rats exposed to 5.8 mg/kg/day) is unclear and might be consistent with male rat-specific precipitation of α_{2F}-globulin (David et al. 2000a). Based on the available data, the kidneys do not demonstrate a consistent response to DEHP.

Endocrine Effects. No information was located regarding endocrine effects in humans after oral exposure to DEHP. Three studies in rats found that DEHP can alter thyroid structure and activity, although the clinical significance of these changes is not clear. Male Wistar rats administered 2,000 mg DEHP/kg/day in the food for 3, 7, or 21 days showed significant reductions in serum thyroxine (T4) levels at all time points, but serum triiodothyronine (T3) levels were essentially unaffected (Hinton et al. 1986). Electron microscopy revealed a considerable increase in the number and size of lysosomes. Also, the Golgi apparatus was enlarged and the mitochondria appeared damaged. These changes were considered indicative of thyroid hyperactivity. Similar changes in the thyroid were reported in a 3-month feeding study, also in male Wistar rats (Price et al. 1988a). In the latter study, the authors observed that in the DEHP-treated rats the colloid was retracted from the follicular cells and contained considerable numbers of basophilic deposits which stained positive for calcium. Although the changes were consistent with thyroid hyperactivity, the rats did not exhibit any clinical signs of hyperthyroidism. A more recent study in Sprague-Dawley rats reported reduced follicle size and colloid density in the thyroid following dietary administration of 345 mg DEHP/kg/day for 13 weeks (Poon et al. 1997).

Other information on endocrine effects following oral exposure to DEHP includes that of no effects on adrenal weight in rats gavaged with up to 1,500 mg/kg/day for 14 days or once with up to 5,000 mg/kg (Berman et al. 1995), and of hypertrophy of anterior pituitary cells in rats administered approximately 674 mg/kg/day for 2 years (Kluwe et al. 1982a).

Further information regarding endocrine effects of DEHP can be found in the following Sections: 3.2.2.5 Reproductive Effects, 3.2.2.6 Developmental Effects, 3.5.2 Mechanisms of Toxicity, and 3.6 Toxicities Mediated Through the Neuroendocrine Axis.

Dermal Effects. No studies were located regarding dermal effects in humans or animals following oral exposure to DEHP

Ocular Effects. No studies were located regarding ocular effects in humans or animals following oral exposure to DEHP

Body Weight Effects. There have been no reports of body weight alterations in humans attributed to oral exposure to DEHP, but numerous studies have documented reductions in body weight gain in animals. Most of the information is on rodents and in general, doses $1,000 mg DEHP/kg administered for periods of 5 days or longer reduce weight gain. Differences between controls and treated animals of >10% in either body weight gain or final weight are considered to be biologically significant by ATSDR. This has been seen after acute-duration exposure in marmoset monkeys (Rhodes et al. 1986), rats (Dostal et al. 1987a, 1987b, 1988; Gray and Butterworth 1980; Mehrotra et al. 1997, 1999; Parmar et al. 1988; Sjoberg et al. 1986a, 1986b), mice (Muhlenkamp and Gill 1998), guinea pigs (Parmar et al. 1988), and rabbits (Parmar et al. 1988). Similar observations have been made in intermediate-duration studies in rats (Barber et al. 1987; Mitchell et al. 1985b; Moccchiutti and Bernal 1997; Parmar et al. 1988; Poon et al. 1997; Shaffer et al. 1945; Short et al. 1987), mice (Ward et al. 1998; Weghorst et al. 1994), guinea pigs (Parmar et al. 1988), and hamsters (Maruyama et al. 1994). No treatment-related changes in body weight were observed in Cynomolgus monkeys that were administered 500 mg DEHP/kg/day by gavage for 14 consecutive days (Pugh et al. 2000), and marmoset monkeys gavaged daily with up to 2,500 mg DEHP/kg for 13 weeks experienced only a 5% reduction in weight gain relative to controls (Kurata et al. 1998). In a chronic study, decreases in weight gain (unquantified) were reported in rats with doses as low as 200 mg/kg/day, but not at 60 mg/kg/day (Carpenter et al. 1953). Other chronic studies in rats reported reductions in weight gain with DEHP doses ranging from 867 to 3,000 mg/kg/day (David et al. 2000a, 2000b; Ganning et al. 1991; Marsman et al. 1988; Rao et al. 1990; Tamura et al. 1990). Dietary exposure to DEHP for 104 weeks caused weight gain reductions of 15% in rats and 9.8% in mice at doses of 789 and 1,266 mg/kg/day, respectively (David et al. 2000a, 2000b). Neither guinea pigs nor dogs administered up to approximately 60 mg DEHP/kg/day for 1 year experienced significant changes in body weight (Carpenter et al. 1953).

Not all studies monitored food consumption and among the ones that did, not all had pair-fed control groups. Therefore, it is unclear whether the reduced weight gain is due solely to reduced food intake or also to reduced food utilization. For example, in one study in rats in which a pair-fed control group was included, DEHP-treated rats had lower weights than the pair-fed controls, even though both groups consumed the same quantities of food (Dostal et al. 1987b). The relevance of these findings to human health is unknown.

Other Systemic Effects. As mentioned above, food consumption has been monitored in a few animal studies during administration of DEHP either by gavage or in the feed. Decreases in food intake were reported in rats (Adinehzadeh and Reo 1998; Dostal et al. 1987b; Mitchell et al. 1985b; Sjoberg et al. 1986a, 1986b). However, it is unclear whether reduced food intake is a reflection of poor palatability of the diets containing DEHP, DEHP-induced decrease in appetite, or both. Thus, the body weight changes should be viewed in the context of a DEHP-induced decrease in food intake.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to DEHP. There were no alterations in spleen and thymus weight or histopathological signs in these organs from rats treated once by gavage with up to 5,000 mg DEHP/kg (Berman et al. 1995). The same results were obtained after the rats were gavaged with up to 1,500 mg DEHP/kg/day for 14 days (Berman et al. 1995). No pathological changes were seen in the spleen during examination of the tissues from rats exposed to up to 1,900 mg DEHP/kg/day for 90 days (Shaffer et al. 1945) or up to 190 mg DEHP/kg/day for 2 years (Carpenter et al. 1953). Similarly, no histological alterations were observed in the spleen, lymph nodes, or bone marrow of F344 rats and B6C3F1 mice that were fed DEHP in the diet at doses #939 or 1,458 mg DEHP/kg/day, respectively, for 104 weeks (David et al. 2000a, 2000b). No study was located that evaluated immune function in animals following exposure to DEHP.

3.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to DEHP. A functional observation battery and motor activity measurements were conducted in Fischer-344 rats before and after a single gavage dose of up to 5,000 mg DEHP/kg or daily gavage doses of up to 1,500 mg/kg/day for 14 days (Moser et al. 1995). The tests assessed autonomic, sensorimotor, and neuromuscular functions as well as excitability and activity. DEHP showed no neurobehavioral toxicity; however, administration of the 5,000 mg/kg dose produced signs of general debilitation. In another study, DEHP had no significant effect on the brain weight in rats or monkeys exposed to 2,000 mg/kg/day for 14 days (Rhodes et al. 1986). No histological alterations were observed in the brain, peripheral and spinal nerves, or spinal cord of F344 rats and B6C3F1 mice that were fed DEHP in the diet at doses #939 or 1,458 mg DEHP/kg/day, respectively, for 104 weeks (David et al. 2000a, 2000b). An effect of DEHP on mammalian nervous tissue was shown in an *in vitro* study in which exposure to DEHP caused increased levels of intracellular Ca^{2+} in rat neurohypophysial nerve terminals and pheochromocytoma

cells (Tully et al. 2000). Only the Moser et al. (1995) data are listed in Table 3-2 and plotted in Figure 3-2, since the end points in the other studies (David et al. 2000a, 2000b; Rhodes et al. 1986) are insufficient indicators of neurotoxicity due to the due to the lack of testing for neurobehavioral function.

3.2.2.5 Reproductive Effects

Testicular Effects. No studies were located regarding reproductive effects in humans after oral exposures to DEHP. Studies in rodents exposed to doses in excess of 100 mg/kg/day DEHP clearly indicate that the testes are a primary target tissue, resulting in decreased testicular weights and tubular atrophy (Dostal et al. 1988; Ganning et al. 1991; Gray and Butterworth 1980; Lamb et al. 1987; Oishi 1994; Parmar et al. 1995; Poon et al. 1997; Saitoh et al. 1997; Shaffer et al. 1945; Sjoberg et al. 1986b). Weights of the seminal vesicles, epididymis, and prostate gland in rats and mice are also reduced by oral exposure DEHP (Gray and Butterworth 1980; Lamb et al. 1987). Within the testis, Sertoli cells appear to be the target of DEHP toxicity (Chapin et al. 1988; Gray and Beamand 1984; Gray and Gangolli 1986; Li et al. 2000; Saitoh et al. 1997; Sjoberg et al. 1986b; Ward et al. 1998). Alterations in Sertoli cells consisting of disruption of the ectoplasmic specializations, including the disappearance of actin bundles in the cells, were seen as early as 3 hours after a single gavage dose of 2,800 mg DEHP/kg in rats (Saitoh et al. 1997). Proliferation of Sertoli cells was reduced and morphology of germ cells was altered (gonocytes were enlarged and multinucleated) 24 hours after administration of a single gavage dose of $100 mg/kg to 3-day-old rat pups (Li et al. 2000). Dietary exposure to DEHP for 90 days caused mild Sertoli cell vacuolation in rats at doses $37.6 mg/kg/day, but not at 3.7 mg/kg/day (Poon et al. 1987). Fertility was reduced in male mice that were exposed to DEHP over a period of 126 days in dietary doses of 140 mg/kg/day, but not 14 mg/kg/day (Lamb et al. 1987), indicating that DEHP affected the process of spermatogenesis. Effects on spermatogenesis were also indicated by the appearance of damaged spermatogenic cells and abnormal sperm in rats exposed to 2,000 mg DEHP/kg/day in the diet for 15 days (Parmar et al. 1987). A study with 2-year-old (prepubertal) Cynomolgus monkeys showed no changes in testes/epididymides weight or testicular histology following treatment with 500 mg DEHP/kg/day by gavage for 14 consecutive days (Pugh et al. 2000).

Biochemical changes in the testes were noted when 2,000 mg/kg/day DEHP was given to rats for 14 days. There was an increase in the activities of gamma-glutamyl transpeptidase (GGT), lactic dehydrogenase, and β-glucuronidase in the treated animals. Conversely, the activities of sorbitol dehydrogenase and acid phosphatase were decreased (Parmar et al. 1987). The significance of these biochemical effects is not clear. More recently, Mehrotra et al. (1997) observed significant reductions in activities of phase II

metabolizing enzymes, in the testis from rats administered approximately 1,740 mg DEHP/kg/day in the diet for 10 days. The most pronounced reduction was that for NAD(P)H quinone oxidereductase, about 50% reduction in activity relative to controls. Mehrotra et al. (1997) suggested that at least part of the testicular toxicity of DEHP might be due to reductions of protective factors against oxidative stress.

There are not enough data to draw conclusions concerning the role that hormones play in the testicular toxicity of DEHP; however, they do appear to have an effect. The coadministration of testosterone with DEHP appeared to diminish but not abolish the testicular toxicity of DEHP in rats (Gray and Butterworth 1980; Parmar et al. 1987). Luteinizing hormone aggravated the testicular toxicity of DEHP in rats (Oishi 1989a).

The age at first exposure to DEHP appears to have a clear influence on the degree and permanence of testicular damage (Dostal et al. 1988; Gray and Butterworth 1980; Gray and Gangolli 1986; Sjoberg et al. 1986b). Tubular structure and spermatogenesis are more affected by prepubertal acute exposure than by postpubertal acute exposure. When newborn rats were exposed to DEHP for 5-day periods at different time intervals between postpartum days 6 and 86, the degree of testicular damage, as manifest in tubular structure, Sertoli cell nuclei, and spermatocytes, decreased as the age of first exposure increased (Dostal et al. 1988; Sjoberg et al. 1986b). When exposures cease prior to sexual maturity, there appears to be an absence of permanence of effect of DEHP on reproductive performance. Rats exposed to DEHP 1 week after birth for 5 days were later successfully mated with control female rats on weeks 8, 10, 11, 12, and 15 (Dostal et al. 1988). There were no significant differences in fertility when the experimental animals were compared to controls.

Testicular zinc levels were measured in all the experimental rats during the Dostal et al. (1988) study. There was no significant change in testicular zinc for any exposure group except the group first exposed when 86 days old. Testicular zinc levels decreased significantly with the 1,000 and 2,000 mg/kg/day doses in these animals. Since zinc is thought to be localized in the spermatids, and the testes from rats younger than 25 days old do not contain spermatids, Dostal et al. (1988) suggested that their results provided further evidence that loss of zinc is not involved in the testicular atrophy at these ages. The role of zinc on DEHP-induced testicular toxicity has been also examined in other studies (Agarwal et al. 1986; Gray and Butterworth 1980; Gray et al. 1982; Oishi and Hiraga 1983), and a more detailed discussion on this issue is presented in Section 3.5.2 Mechanisms of Toxicity.

With chronic exposures to DEHP, the testicular damage persists, as was demonstrated by inhibition of spermatogenesis and atrophy seen in rats after lifetime exposure to 600–2,000 mg/kg/day (David et al. 2000a; Kluwe et al. 1982a; Price et al. 1987). In the David et al. (2000a) study, 6-week-old male rats were fed diets that provided 0, 5.8, 29, 147, or 789 mg DEHP/kg body weight/day for 104 weeks. Testicular effects included significantly (p#0.05) increased incidences of bilateral aspermatogenesis at $29 mg/kg/day and reduced testes weight (63% absolute, 59% relative) at 789 mg/kg/day. Castration cells in the pituitary, apparently due to reduced testosterone secretion resulting from an effect of DEHP on the Sertoli cells, were also observed in the rats exposed to 789 mg/kg/day. Castration cells are vacuolated basophilic cells in the anterior pituitary gland usually observed after castration. The percentage of rats with aspermatogenesis was 58, 64, 78, 74, and 97% in the control to high dose groups, respectively. Because there is a clear dose-related increase in aspermatogenesis and the effect is consistent with the reduced testes weights observed at the highest dose, the NOAEL and LOAEL for testicular toxicity are 5.8 and 29 mg/kg/day, respectively (NTP 2000b). Using the 5.8 mg/kg/day testicular NOAEL, a chronic oral MRL of 0.06 mg/kg/day was derived for DEHP as discussed in Section 2.3 and detailed in Appendix A. Testicular effects (inhibition of spermatogenesis and general tubular atrophy) were also reported at dietary doses as low as 14 mg/kg/day in a 102-week rat study (Ganning et al. 1991), but assessment of the results is complicated by a lack of incidence data.

In mice exposed to 140 and 420 mg/kg/day DEHP for 126 days in a continuous breeding experiment, there was a significant decrease in weights of the testes, epididymis, and prostate gland (Lamb et al. 1987). The numbers of motile sperm and the sperm concentration were decreased significantly, and there was an increase in the number of abnormal sperm. The NOAEL for reduced fertility in this study, 14 mg/kg/day, was used to derive an intermediate oral MRL of 0.1 mg/kg/day for DEHP as discussed in Chapter 2 and detailed in Appendix A. When the high dose (420 mg/kg/day) males were mated with unexposed females, significantly fewer litters were produced. The number of pups born alive and pup body weights were also significantly lower than the controls (Lamb et al. 1987). Although 140 mg/kg/day is the lowest LOAEL for effects on reproductive function (Table 3-2), this dose might not represent a true LOAEL because development of the reproductive system and reproductive performance in the second generation were not evaluated, and testicular toxicity has occurred at lower doses ($37.6 mg/kg/day) in other intermediate-duration studies in rats (Parmer et al. 1995; Poon et al. 1997). Testicular effects observed in mice exposed to DEHP in the diet for 2 years included decreased testes weights and increased incidence and severity of bilateral hypospermia and immature/abnormal sperm in the epididymis at $292 mg/kg/day (David et al. 2000b), and seminiferous tubular degeneration at 1,325 mg/kg/day (Kluwe et al. 1982a).

Development of the reproductive system was adversely affected in male offspring of rats that were exposed to DEHP at 1,060 mg/kg/day, but not #339 mg/kg/day, for 70 days prior to mating through gestation and lactation in a two-generation dietary study (Schilling et al. 1999). Effects observed in this study included reduced testes and epididymis weights, testicular lesions, and spermatocyte loss in F_1 males, reduced postnatal survival in F_1 pups and reduced prenatal survival in F_1 and F_2 pups, and altered sexual differentiation in F_1 males (increased nipple retention) and F_2 males (reduced anogenital distance). Similar effects on the male reproductive system have been observed in developmental toxicity studies at lower doses (see Section 3.2.2.6 Developmental Effects). These studies provide evidence that abnormal development and formation of the male reproductive system are likely to be the most sensitive reproductive end points for DEHP.

Female Fertility. Few studies of the reproductive effects of DEHP on females have been conducted. However, based on the existing data, long-term exposures of females also appear to have deleterious effects. When female mice were exposed to dietary doses of 140 mg/kg/day DEHP for 126 days and mated with control males, no litters were produced (Lamb et al. 1987). The combined weights of the ovaries, oviducts, and uterus were significantly lower than those for the controls. A more recent study in rats showed that DEHP short-term treatment with 2,000 mg DEHP/kg by gavage decreases serum estradiol levels resulting in increased serum FSH levels and absence of LH surges necessary for ovulation (Davis et al. 1994a). Thus, exposure to DEHP resulted in hypoestrogenic anovulatory cycles and polycystic ovaries in adult female rats. In a study that evaluated the estrogenic activity of DEHP and other phthalate esters, DEHP induced no reproducible significant increases in uterine wet weight in immature ovariectomized rats and did not affect the degree of vaginal epithelial cell cornification in mature ovariectomized rats (Zacharewski et al. 1998). Results from *in vitro* estrogenicity assays conducted in that study as well as in other studies are summarized in Section 3.5 Toxicities Mediated Through the Neuroendocrine Axis.

Nonrodent Species. Few reproductive studies of DEHP have been conducted in nonrodent species. A dose of 2,000 mg/kg/day given to 12- to 18-month-old marmoset monkeys for a 14-day period had no effect on testicular weight (Rhodes et al. 1986). No changes in testes/epididymides weight or testicular histology occurred in 2-year-old Cynomolgus monkeys that were treated with 500 mg DEHP/kg/day by gavage for 14 consecutive days (Pugh et al. 2000). A 13-week gavage study in marmosets of unspecified age showed no significant treatment-related effects (up to 2,500 mg DEHP/kg/day) on gross or microscopical appearance of the testis or in testicular zinc content, or on gross or microscopical

appearance of the uterus, vagina, or ovary (Kurata et al. 1998). These studies suggest that nonhuman primates are less sensitive than rodents to the effects of DEHP using these end points of toxicity.

Responsible Metabolite. Since DEHP is metabolized to a variety of compounds following oral exposure, the question has been raised as to which of DEHP metabolites is responsible for the testicular effects of DEHP. Evidence suggests that mono(2-ethylhexyl) phthalate (MEHP) might be the toxic metabolite in the testes. In one study, 1,055 mg/kg/day of DEHP administered for 5 days to rats did not affect testicular weight or structure, but an equimolar dose of MEHP had a significant effect (Sjoberg et al. 1986a). Further information on the testicular toxicity of DEHP/MEHP is presented in Section 3.5.2 Mechanisms of Toxicity.

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No studies were located regarding developmental toxicity in humans after oral exposure to DEHP. DEHP has been demonstrated to cause developmental toxicity including teratogenic effects in both rats and mice. Effects observed included decreased fetal/pup body weight, increased rates of abortion and fetal resorptions, or malformations. Single very large doses of 4,882 and 9,756 mg/kg DEHP administered to pregnant Wistar rats on day 12 of gestation caused a dose-related increase in dead and resorbed fetuses and a number of malformations in the survivors (Ritter et al. 1987). The types of malformations observed in the newborn pups included hydronephrosis, cardiovascular malformations, and tail malformations. Repeated doses of 1,000 mg/kg on gestation days 6–15 increased the incidence of fetal deaths and of external, soft tissue, and skeletal malformations in offspring from Wistar rats (Hellwig et al. 1997); doses of 200 mg DEHP/kg had no significant effects. Administration of 1,700 mg DEHP/kg/day to Wistar rats for 90 days prior to confirmed pregnancy with no further dosing decreased fetal weight by 10% and placental weight by 8%, but it did not significantly increase the incidence of malformations relative to controls (Nikonorow et al. 1973). Dietary administration of DEHP to Fischer-344 rats throughout pregnancy caused increased percentages of resorptions and dead pups per litter at doses as low as 313 mg/kg/day (Price et al. 1986; Tyl et al. 1988). There was no conclusive evidence of teratogenic malformations at doses #1,055 mg/kg/day (Tyl et al. 1988), and offspring of rats that were exposed to #573 mg/kg/day had no effects on growth and viability after postnatal day 4, age of acquisition for

developmental landmarks (incisor eruption, wire grasping, eye opening, testes descent, or vaginal opening), spontaneous locomotor activity, or reproductive performance (Price et al. 1986).

Mice appear to be more susceptible to the developmental effects of DEHP than rats, at least when DEHP is given in the diet and comparisons are limited to traditional teratogenicity studies (i.e., studies that are not specifically designed to detect male reproductive system abnormalities). Decreased fetal viability and increased resorptions and external malformations were observed in ICR mice gavaged with 1,000 mg DEHP/kg/day on gestation days 7–9 and examined on gestation day 18 (Shiota and Mima 1985); the NOAEL was 500 mg/kg/day. Defects of the anterior neural tube were the most commonly observed malformations. Experiments conducted by Yagi et al. (1980) and Tomita et al. (1982a), in which pregnant ddY-Slc mice were administered a single gavage dose of DEHP of gestation days 6, 7, 8, 9, or 10 and killed on day 10, showed that the most severe effects occurred when the DEHP was given on gestation day 7. Dosing on day 7 with 100 mg DEHP/kg/day produced an 11% incidence of fetal lethality compared to 2.5% at 50 mg/kg and 0% in controls (Tomita et al. 1982a). The 11% rate was similar to that seen when dosing on day 9 with 7,500 mg/kg or on day 6 with 2,500 mg/kg. Doses of approximately 170 mg DEHP/kg administered to ICR mice in the food on gestation days 1–18 significantly increased the percent of resorptions and dead fetuses (Shiota et al. 1980); all implanted ova died *in utero* at 683 mg/kg/day. The NOAEL was 83 mg/kg/day. A significant increase in malformations of the external viscera and skeleton was apparent in CD-1 mice at doses of 91, 191, and 292 mg/kg/day given throughout gestation (Tyl et al. 1988). Specific abnormalities observed included protrusion of the eyeball, exencephaly, blood vessel abnormalities, fused or branched ribs, misaligned and fused thoracic vertebrae, and tail malformations. Maternal and fetal toxicity were reflected in the body weight data. No adverse effects were seen at a dose of 44 mg/kg/day. Prenatal and perinatal mortality was increased in offspring of mice that were exposed to 95 mg/kg/day DEHP in the diet on gestation days 0–17, although there were no effects on growth and viability after postnatal day 4, age of acquisition for developmental landmarks, spontaneous locomotor activity, or reproductive performance (Price et al. 1988c).

Since DEHP is a lipophilic substance, it has the potential to accumulate in maternal milk and be transferred to suckling offspring. Both DEHP (216 µg/mL) and MEHP (25 µg/mL) were detected in the milk collected from lactating Sprague-Dawley rats 6 hours after the last of three daily gavage doses of 2,000 mg DEHP/kg (Dostal et al. 1987b). At this time, no DEHP could be detected in dams' plasma, but substantial amounts of MEHP were detected. Pups' plasma had no detectable DEHP or MEHP. No other dose levels were tested in this study. Dostal et al. (1987b) noted that exposure to DEHP was associated with an increase in milk solids and lipids. Addition of ^{14}C-DEHP to milk *in vitro* resulted in 94% of the

radioactivity associated with the fat globular layer, 4% was in the whey, and 1.6% was in the casein pellet.

Developmental effects of DEHP in rats exposed via maternal milk have been studied. Treatment of Sprague-Dawley rats with 2,000 mg DEHP/kg/day on postpartum days 2–6, 6–10, or 14–18 reduced pup weight and induced palmitoyl-CoA and carnitine acetyltransferase in the pups liver (Dostal et al. 1987b). Administration of 500 mg DEHP/kg/day to Fischer-344 rats on postpartum days 1–21 caused approximately a 24% reduction in pup weight on postnatal day 21 (Cimini et al. 1994). All pups from groups administered 2,500 or 5,000 mg DEHP/kg/day died before postnatal day 12. The 5,000 mg/kg/day dose caused 25% lethality in the dams. Results from peroxisome enzyme activities evaluation (only 1,000 mg/kg/day group) in 14-day-old pups liver, kidneys, and brain showed a doubling of catalase specific activity in the liver and kidneys, doubling of D-amino acid oxidase activity in the three organs, and of dihydroxyacetone phosphate acyltransferase in the liver. Palmitoyl-CoA oxidase was greatly induced in the liver, to a lesser extent in the kidneys, and only slightly in the brain. In general, changes in enzyme activity were reversible with time courses dependent on the enzyme and tissue. Electron microscopy showed peroxisome proliferation in both liver and kidneys, but no information was reported about the brain (Cimini et al. 1994). Peroxisome proliferation in the brain from pups exposed via breast milk from dams treated with 2,800 mg DEHP/kg/day had been reported earlier (Dabholkar 1988). A significant observation of Cimini et al. (1994) was the fact that brain catalase activity doubled in adults dams treated with DEHP, but was not significantly changed in the pups. Results similar to those of Cimini et al. (1994) were reported by Stefanini et al. (1995).

Gestational and lactational exposure to DEHP has been shown to profoundly alter reproductive system development in male rat offspring. Administration of 750 mg DEHP/kg/day by gavage from gestation day 14 to postnatal day 3 induced a variety of effects in androgen-dependent tissues in neonates and infant pups at 3–7 months of age, including reduced anogenital distance (female rats normally have a shorter anogenital distance than males), permanent female-like areolas and nipples, vaginal pouch formation, penile abnormalities (e.g., cleft phallus with hypospadias), hemorrhagic and undescended testes, testicular and epididymal atrophy or agenesis, and small to absent sex accessory glands (e.g., seminal vesicles and ventral prostate) (Gray et al. 1999, 2000; Parks et al. 2000). Dose levels other than 750 mg/kg/day were not tested in these studies. Similar effects were observed in male offspring of rats that were exposed to 375, 750, and/or 1,500 mg DEHP/kg/day from gestation day 3 through postnatal day 21 as discussed below (Moore et al. 2001). Abnormalities that were dose-related included reduced anogenital distance, areola and nipple retention, undescended testes, permanently incomplete preputial

separation, and greatly reduced anterior prostate weight. Most of the effects were observed at $750 mg/kg/day, although permanent areola/nipple retention and reduced anterior prostate weight also occurred at 375 mg/kg/day, indicating that this is the LOAEL for effects on male reproductive system development. The percentage of litters with major reproductive defects (defined as litters with at least one male with missing or severely malformed sex organs, one testis or epididymis that weighed <75% of the other, incomplete preputial separation, or an undescended testis in adulthood) were 0, 62.5, 87.5, and 100% at 0, 375, 750, and 1,500 mg/kg/day, respectively. Behavioral observations indicated that many of the exposed males were sexually inactive in the presence of receptive control females, although sexual inactivity did not correlate with abnormal male reproductive organs. Evaluation of the behavioral data is limited by small numbers of animals; however, a total lack of sexual activity by three of seven males at 375 mg/kg/day suggests the possibility that exposure to the LOAEL can also demasculinize sexual behavior.

Developmental toxicity was observed in offspring of Long-Evans rats that were exposed to DEHP in the drinking water at reported estimated doses of 3.3 or 33 mg/kg/day throughout pregnancy and continuing during postnatal days 1–21 (Arcadi et al. 1998). Examinations of the pups on postnatal days 21–56 showed effects that included severe testicular histopathological changes at both DEHP dose levels, particularly at 21 and 28 days of age. Alterations consisted of gross disorganization of the seminiferous tubules with detachment of the spermatogonial cells from basal membrane and absence of spermatocytes. These changes were still present in high-dose rats at 56 days of age; at this age, low-dose rats exhibited only a few elongated spermatides, whereas unexposed rats had fully developed tubular structure with complete spermatogenesis. The effect levels in this study are not considered to be reliable and are unsuitable for identifying a LOAEL and for use in MRL derivation because (1) the methods used to verify and characterize the administered doses were not clearly described or completely reported, and could not be resolved by the NTP Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) Expert Panel on DEHP, and (2) the authors did not put their blood DEHP concentration data into context with other studies (NTP 2000b).

The morphological changes to the male reproductive system, as well as the male sexual behavioral observations, are consistent with an antiandrogenic action of DEHP. Other indications of antiandrogenic activity include a lack of significant effects on time to vaginal opening and first estrus in female offspring of the rats that were exposed to 375–1,500 mg DEHP/kg/day from gestation day 3 through postnatal day 21 (Moore et al. 2001). Additionally, exposure to 750 mg/kg/day from gestation day 14 to postnatal day 3 caused significantly reduced testicular testosterone production and testicular and whole-body

testosterone levels in fetal and neonatal males (Parks et al. 2000). Differences in whole-body testosterone levels were greatest at gestation day 17 (71% lower than controls). As a consequence, anogenital distance on postnatal day 2 was reduced 36% in exposed male, but not female, offspring, and testis weight was significantly reduced at gestation day 20 and postnatal day 2 (18.6 and 49.4% lower than controls, respectively). Additional information on the antiandrogenic action of DEHP is discussed in Section 3.5.2 (Mechanisms of Toxicity).

Several studies have focused on identifying the active developmental toxicant. For example, in a gavage study in Wistar rats, on an equimolar basis, DEHP was less teratogenic than MEHP, which in turn was less teratogenic than 2-ethylhexanoic acid (Ritter et al. 1987). In ICR mice, gavage administration of DEHP caused more severe embryotoxicity and teratogenicity than MEHP (Shiota and Mima 1985). In Han:NMRI mice, the (R) enantiomer of 2-ethylhexanoic acid given intraperitoneally was highly teratogenic or embryotoxic, no such properties were seen for the (S) enantiomer (Hauck et al. 1990). In rabbits, 2-ethylhexanoic acid did not cause developmental effects even at oral doses (125 and 250 mg/kg/day) that were maternally toxic (Tyl 1988b).

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

No studies were located regarding cancer in humans after oral exposure to DEHP. However, several chronic feeding studies in rodents indicate that DEHP can cause liver tumors in rats and mice (David et al. 1999; Kluwe et al. 1982a; Rao et al. 1987, 1990). For example, a clear dose-dependent increase in the incidence of hepatocellular adenomas was noted in male/female mice fed 672/799 and 1,325/1,821 mg/kg/day and male/female rats fed 322/394 and 674/774 mg/kg/day for 2 years (Kluwe et al. 1982a; NTP 1982). With a dose of 3,000 mg/kg/day, 78.5% of the treated rats developed liver tumors and 29% pancreatic islet cell adenomas (Rao et al. 1990). These findings have been criticized on the basis that the doses given exceed the maximum tolerated dose and that liver tumors are common in control animals of these species (Northup et al. 1982). Hayashi et al. (1994) also observed a significant increase in the incidence of hepatocarcinomas in Fischer-344 rats fed a diet that provided approximately 1,580 mg DEHP/kg/day for up to 78 weeks. However, no hepatocarcinomas or nodules were seen at week 52. In DEHP-treated rats, peroxisomal β-oxidation activities increased 11–15-fold during the experimental period, but catalase activity increased only 2-fold after 10 weeks of treatment and by 30–78 weeks had

gradually decreased. After 30 weeks, there was no indication of oxidative damage or of oxidative cytotoxicity.

The relationship between hepatic peroxisome proliferation, cell proliferation, and carcinogenicity has been evaluated in chronic studies of DEHP in rats and mice (David et al. 1999, 2000a, 2000b). Animals were fed a diet containing DEHP for 104 weeks; additional groups treated for only 78 weeks were subsequently placed in a DEHP-free diet for 26 weeks to examine reversibility of the effects. In rats, relative liver weight was significantly increased at $147 mg/kg/day and this correlated with increased palmitoyl CoA oxidase activities. Treatment with DEHP resulted in hepatocellular adenomas and carcinomas at weeks 78 and 104; in general, the incidence of tumors at week 78 was low except for the high-dose group. Cessation of treatment resulted in a one-third to one-half decrease in the incidence of total neoplasia relative to the groups treated for 104 weeks and in a reduction in liver weight and palmitoyl Co-A oxidase activity. The increased incidence of hepatic tumors was significant at the 147 mg/kg/day dose level and higher in rats. Similar findings were reported in B6C3F1 mice. Cessation of treatment in mice resulted in a 50% decrease in the incidence of total neoplasia in males relative to groups treated for 104 weeks, but the incidence in females decreased only slightly. The increased incidence in hepatocellular tumors achieved statistical significance at a dose levels of $292 mg DEHP/kg/day.

DEHP did not induce forestomach tumors in ICR mice administered approximately 1,171 mg DEHP/kg/day 2 days/week for 4 weeks and sacrificed after 22 weeks of treatment (Lee et al. 1997). Also in this study, DEHP had no significant effect on the number of forestomach tumors/mouse induced by treatment with benzo[a]pyrene once per week for 4 weeks. Initiation-promotion studies and the role of promotion in the carcinogenicity of DEHP are discussed in Section 3.5.2 Mechanisms of Toxicity.

All Cancer Effect Level (CEL) values for rats and mice in the chronic-duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Based on the increased incidence of hepatocellular carcinoma and adenoma in male mice (NTP 1982), EPA calculated an oral slope factor of 1.4×10^{-2} $(mg/kg/day)^{-1}$ (IRIS 2000). Based on this value, oral intakes of 7.1×10^{-3} to 7.1×10^{-6} mg/kg/day correspond to excess cancer risk levels of 10^{-4} to 10^{-7}, respectively. Figure 3-2 shows this range of human upper bound risk values.

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding lethality in humans after dermal exposure to DEHP. When rabbits were exposed to single doses of up to 19,800 mg/kg DEHP using a modification of the FDA cuff test, two of six rabbits in the highest dose group died. The dermal LD_{50} value calculated from these data was 24,750 mg/kg (Shaffer et al. 1945).

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, or renal effects in humans or animals after dermal exposure to DEHP.

Dermal Effects. An unidentified quantity of DEHP was applied to the skin of 23 humans for a 7-day period without adverse effect. After 10 days without exposure, DEHP was applied to the same spot on each subjects' back. No adverse reactions were observed during either phase of the study indicating that DEHP is neither a dermal irritant, nor a sensitizer in humans (Shaffer et al. 1945).

Single doses of up to 19,800 mg/kg DEHP were applied to rabbit skin using a modified FDA cuff test procedure. There was no evidence of dermal irritation caused by DEHP during the 14-day observation period.

Ocular Effects. There was no necrosis of rabbit cornea after ocular exposure to a single dose of 0.5 mL (495 mg) DEHP but a slight transient reddening of the eyelids was observed (Shaffer et al. 1945). These data indicate that neat DEHP does not act as a dermal or ocular irritant in rabbits.

No studies were located regarding the following health effects in humans or animals after dermal exposure to DEHP:

3.2.3.3 Immunological and Lymphoreticular Effects

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.3 GENOTOXICITY

No studies were located regarding genotoxic effects in humans after inhalation, oral, or dermal exposure to DEHP. As discussed below, DEHP has been extensively tested in a variety of short-term genotoxicity assays with predominantly negative or false-positive results. The weight of evidence from these assays, as well as the tumor initiating/promoting activity studies summarized in Section 3.5.2 (Mechanisms of Toxicity), indicate that DEHP does not induce lesions in nuclear DNA, is not mutagenic/genotoxic, and is not a tumor initiator, but rather that it is a rodent liver mitogen and tumor promoter, and is best characterized as an epigenetic toxicant.

Mammalian *in vivo* genotoxicity studies are summarized in Table 3-3. Most of these assays found that DEHP is not genotoxic. Binding of DEHP to DNA in rat liver was reported by Albro et al. (1982a, 1982b), but was not observed by other investigators (Gupta et al. 1985; Lutz 1986; Von Däniken et al. 1984). 8-Hydroxydeoxyguanosine was detected in hepatic DNA from rats exposed to 1,200 mg/kg/day DEHP for 2 weeks (Takagi et al. 1990). Production of 8-hydroxydeoxyguanosine is a potential marker of genotoxicity; however, many chemicals that induced this lesion in total liver DNA have not been shown to be mutagenic, tumor initiators, or complete carcinogens (Trosko 2001; Trosko et al. 1998; Yakes and van Houten 1997).

DEHP tested negative in mouse bone marrow micronucleus and rat liver DNA repair assays *in vivo* (Cattley et al. 1988; Putman et al. 1983). DNA synthesis increased in rats immediately following DEHP exposure due to increased cell division (Mitchell et al. 1985b; Smith-Oliver and Butterworth 1987), and rats that were exposed to 1,000 mg/kg/day DEHP for periods of 3 or 7 days alternating with 7-day withdrawal periods had increased liver cell division and numbers of tetraploid nuclei during the exposure periods (Ahmed et al. 1989). During the withdrawal periods in the latter study, the cell number declined and degenerated cells appeared to be those containing the tetraploid nuclei. Cells are more vulnerable to irreversible mutagenic alterations during a period of rapid cell division (Marx 1990), and it has been postulated that the carcinogenicity of DEHP might be a consequence of its induction of cell division in

the liver in the presence of other mutagens (Smith-Oliver and Butterworth 1987). The evidence supports the interpretation that DEHP is mitogenic, not mutagenic, because mutagens, by inducing DNA lesions, would inhibit DNA synthesis and cell proliferation.

Increased activity of liver poly (ADP-ribose) polymerase (pADPRP), an effect related to DNA repair, cell proliferation, and differentiation, was observed in rats treated orally with DEHP by gavage for 7 days or in the food for up to 97 weeks (Hayashi et al. 1998). Dominant lethal mutations were increased in mice that were exposed to DEHP by injection at dose levels that also resulted in decreased fertility, but not by oral administration (Autian 1982; Rushbrook et al. 1982; Singh et al. 1974). The results of these studies are not necessarily indicative of genotoxicity because DEHP has not been shown to induce DNA lesions in most studies and positive findings can be interpreted in different ways. For example, dominant lethal tests can be interpreted as indicating that the test chemical altered gene expression (i.e., by epigenetically shut off the marker gene) rather than by mutation.

Table 3-3. Genotoxicity of DEHP *In Vivo*

Species (tests system)	End point	Results	Reference
Mammalian cells:			
Human leucocytes	DNA damage	+	Anderson et al. 1999
Human leucocytes	Chromosomal aberrations	–	Thiess and Fleig 1978
Hamster embryo cells	Chromosomal aberrations	+	Tomita et al. 1982b
Hamster embryo cells	Cell transformation	+	Tomita et al. 1982b
Hamster embryo cells	8AG/6TG-resistant mutation	+ (?)	Tomita et al. 1982b
Rat bone marrow	Micronuclei	–	Putman et al. 1983
Rat bone marrow	Mitotic index	–	Putman et al. 1983
Mouse	Dominant lethal test	–	Rushbrook et al. 1982
Mouse	Dominant lethal test	+	Autian 1982
Mouse	Dominant lethal test	+	Singh et al. 1974
Mouse bone marrow	Micronuclei	–	Astill et al. 1986
Mouse bone marrow	Micronuclei	–	Douglas et al. 1986
Mouse bone marrow	Micronuclei	–	Putman et al. 1983
Rat liver	DNA binding	+	Albro et al. 1982a
Rat liver	DNA binding	–	Gupta et al. 1985
Rat liver	DNA binding	–	Lutz 1986
Rat liver	DNA binding	–	Von Däniken et al. 1984
Rat liver	DNA repair	–	Butterworth et al. 1984
Rat liver	DNA repair	–	Cattley et al. 1988
Rat liver	DNA repair	–	Kornbrust et al. 1984
Rat liver	DNA repair	+	Hayashi et al. 1998

Table 3-3. Genotoxicity of DEHP *In Vivo (continued)*

Species (tests system)	End point	Results	Reference
Mouse liver	DNA repair	–	Smith-Oliver and Butterworth 1987
Rat liver	Strand breaks	–	Butterworth et al. 1984
Rat liver	Strand breaks	–	Elliott and Elcombe 1985
Rat liver	Strand breaks	–	Tamura et al. 1991
Rat liver	DNA base modification	–	Cattley and Glover 1993
Rat liver	DNA base modification	+	Takagi et al. 1990
Rat liver	Tetraploid nuclei	+	Ahmed et al. 1989
Rat kidney	Tumor promotion	+	Kurokawa et al. 1982
Host-meditated assay:			
S. typhimurium (TA100) Rat host-meditated	Gene mutation	–	Kozumbo et al. 1982
Eukaryotic organisms:			
Drosophila (injection)	Sex linked recessive lethal	–	Yoon et al. 1985

– = negative result; + = positive result; ATPase = adenosine triphosphatase; GGTase = gamma glutamyl transferase

Spot tests were conducted in which mouse embryos heterozygous for a number of recessive coat color mutations were exposed *in utero* with the direct monofunctional alkylating mutagen ethylnitrosourea (ENU), either alone or followed by intraperitoneal injection of the pregnant dam with DEHP (Fahrig and Steinkamp-Zucht 1996). DEHP, in combination with ENU, resulted in an increase in the number of spots indicative of reciprocal recombination compared to ENU treatment alone. Conversely, DEHP alone resulted in a reduction in the number of spots that arose from ENU-induced gene mutations. These findings are not necessarily indicative of interference with DNA repair processes because DEHP could have induced altered spots epigenetically rather than by mutagenic means. As discussed by Trosko (1997, 2001), mutation assays are often misinterpreted to give false positives results for epigenetic (nonmutagenic) agents.

DEHP has been extensively tested in short-term *in vitro* genotoxicity assays, but does not appear to be mutagenic in most microbial and mammalian assay systems, as evident in the study results summarized in Table 3-4. The International Program on Chemical Safety (IPCS) investigated the *in vitro* genotoxicity of DEHP in a comprehensive study (IPCS 1985). They found no evidence for genotoxicity in standard bacterial tests. Likewise, there was no evidence for genotoxicity expressed as strand breaks, sister chromatid exchanges, chromosomal aberrations, micronuclei, or polyploidy in mammalian systems *in vitro*. Potential for mutagenicity in mammalian systems *in vitro* was suggested in only 1/10 assays, 2/4 for DNA repair, 4/5 for cell transformation, 2/2 for aneuploidy, and 1/2 for metabolic cooperation. Genotoxicity tests using fungi yielded positive results in 1/7 assays for mutations, 3/7 (gene conversion), 1/6 (crossing-over), and 2/4 (aneuploidy). The few positive test results from the IPCS (1985) study and the studies of other investigators (Table 3-4) were typically associated with limitations that included weak or nonreproducible positive responses, limited experimental conditions, positive responses only at restricted or cytotoxic dose levels, and lack of quantitative evaluation. Induction of cell transformation (Diwan et al. 1985; IPCS 1985; Mikalsen et al. 1990; Sanner and Rivedal 1985) and aneuploidy (IPCS 1985; Stenchever et al. 1976) provided the clearest indications of genotoxic potential, but false positive interpretations are not precluded.

Table 3-4. Genotoxicity of DEHP *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium	Gene mutation	–	–	Astill et al. 1986
S. typhimurium	Gene mutation	–	–	Barber et al. 1987
S. typhimurium	Gene mutation	–	–	Kirby et al. 1983
S. typhimurium (TA100)	Gene mutation	–	+	Kozumbo et al. 1982
S. typhimurium (TA98)	Gene mutation	–	–	Sato et al. 1994
S. typhimurium (TA102)	Gene mutation	–	–	Schmezer et al. 1988
S. typhimurium (TA100)	Gene mutation	–	–	Seed 1982
S. typhimurium	Gene mutation	–	–	Tennant et al. 1987
S. typhimurium (TA100)	Gene mutation	+		Tomita et al. 1982b
S. typhimurium (TA98, TA100)	Gene mutation	–	–	Yoshikawa et al. 1983
Escherichia coli PQ37	Gene mutation	–	–	Sato et al. 1994
E. coli WP2UVRA$^+$	Gene mutation	–	–	Yoshikawa et al. 1983
E. coli WP2UVRA	Gene mutation	–	–	Yoshikawa et al. 1983
Bacillus subtilis (*rec* assay)	DNA damage	–	–	Tomita et al. 1982b
S. typhimurium	Azaguanine resistance	–	–	Seed 1982

Table 3-4. Genotoxicity of DEHP *In Vitro (continued)*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Eukaryotic organisms:				
Saccharomyces cerevisae (XV185-14C, D7, RM52, D6, D5, D6-1)	Gene mutation	–	–	Parry et al. 1985
S. cerevisiae (JD1, D7-144, D7)	Gene conversion	–	–	Parry et al. 1985
S. cerevisiae (D61M, D6)	Mitotic aneuploidy	+	+	Parry et al. 1985
S. cerevisiae (D61M, D6)	Mitotic segregation	–	–	Parry et al. 1985
Schizosaccharomyces pombe (P1)	Gene mutation	–	–	Parry et al. 1985
Aspergillus niger (P1)	Mitotic segregation	–	NS	Parry et al. 1985
Mammalian cells:				
Mouse lymphoma cells	Mutagenicity	–	–	Astill et al. 1986
Mouse lymphoma cells	Mutagenicity	–	–	Kirby et al. 1983
Mouse lymphoma cells	Mutagenicity	–	–	Tennant et al. 1987
Rat hepatocytes	DNA damage	–	NA	Schmezer et al. 1988
Hamster hepatocytes	DNA damage	–	NA	Schmezer et al. 1988
Human hepatocytes	DNA repair	–	NA	Butterworth et al. 1984
Mouse hepatocytes	DNA repair	–	NA	Smith-Oliver and Butterworth 1987
Rat hepatocytes	DNA repair	–	NA	Astill et al. 1986
Rat hepatocytes	DNA repair	–	NA	Butterworth 1984
Rat hepatocytes	DNA repair	–	NA	Hodgson et al. 1982
Rat hepatocytes	DNA repair	–	NA	Kornbrust et al. 1984

Table 3-4. Genotoxicity of DEHP *In Vitro (continued)*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Rat hepatocytes	DNA repair	–	NA	Probst and Hill 1985
V79 cells	DNA repair	–	NA	Kornbrust et al. 1984
CHO cells	Sister chromatid exchange	–	NA	Abe and Sasaki 1977
CHO cells	Sister chromatid exchange	–	NA	Phillips et al. 1982
CHO cells	Sister chromatid exchange	+	NA	Tennant et al. 1987
Rat liver (RL4)	Sister chromatid exchange	–	NA	Priston and Dean 1985
Human hepatocytes	Chromosomal aberrations	–	NA	Turner et al. 1974
Human leucocytes	Chromosomal aberrations	–	NA	Stenchever et al. 1976
CHO cells	Chromosomal aberrations	–	NS	Phillips et al. 1982
CHO cells	Sister chromatid exchange	–	NA	Tennant et al. 1987
Rat liver (RL4)	Chromosomal aberrations	–	NA	Priston and Dean 1985
CH SV40-transformed liver cells	Selective DNA amplification	–	NA	Schmezer et al. 1988
CHO cells	Cell transformation	+	NS	Sanner and Rivedal 1985
SHE cells	Cell transformation	–	NA	Astill et al. 1986
Mouse JB6 epidermal cells	Cell transformation	+	NA	Diwan et al. 1985
SHE cells	Cell transformation	+	NA	Mikalsen et al. 1990
Mouse C3H/10T1/2 fibroblasts	Cell transformation	–	NA	Sanchez et al. 1987

Table 3-4. Genotoxicity of DEHP *In Vitro (continued)*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Chinese hamster fibroblasts	Gap junction intercellular communication	+	NS	Malcolm and Mills 1989
Chinese hamster fibroblasts	Gap junction intercellular communication	–	NS	Kornbrust et al. 1984
Rat hepatocytes	DNA binding	–	NA	Gupta et al. 1985
Human fetal pulmonary cells	Aneuploidy	–	NA	Stenchever et al. 1976
Rat liver (RL4)	Polyploidy	–	NA	Priston and Dean 1985

– = negative result; + = positive result; CHO = Chinese hamster ovary; NA = not applicable to mammalian cell cultures; NS = not specified; SHE = Syrian hamster embryo

3.4 TOXICOKINETICS

Human data indicate that gastrointestinal absorption of DEHP and its metabolites might amount to approximately 20–25% of an orally-administered dose. Trace amounts of DEHP might be absorbed through the skin. Parenteral routes of exposure are also a human concern since DEHP is found in plastic products that might be used in medical treatment devices or storage bags. No human data were available regarding the toxicokinetics of inhaled DEHP, although some degree of absorption from respiratory tissues would be expected.

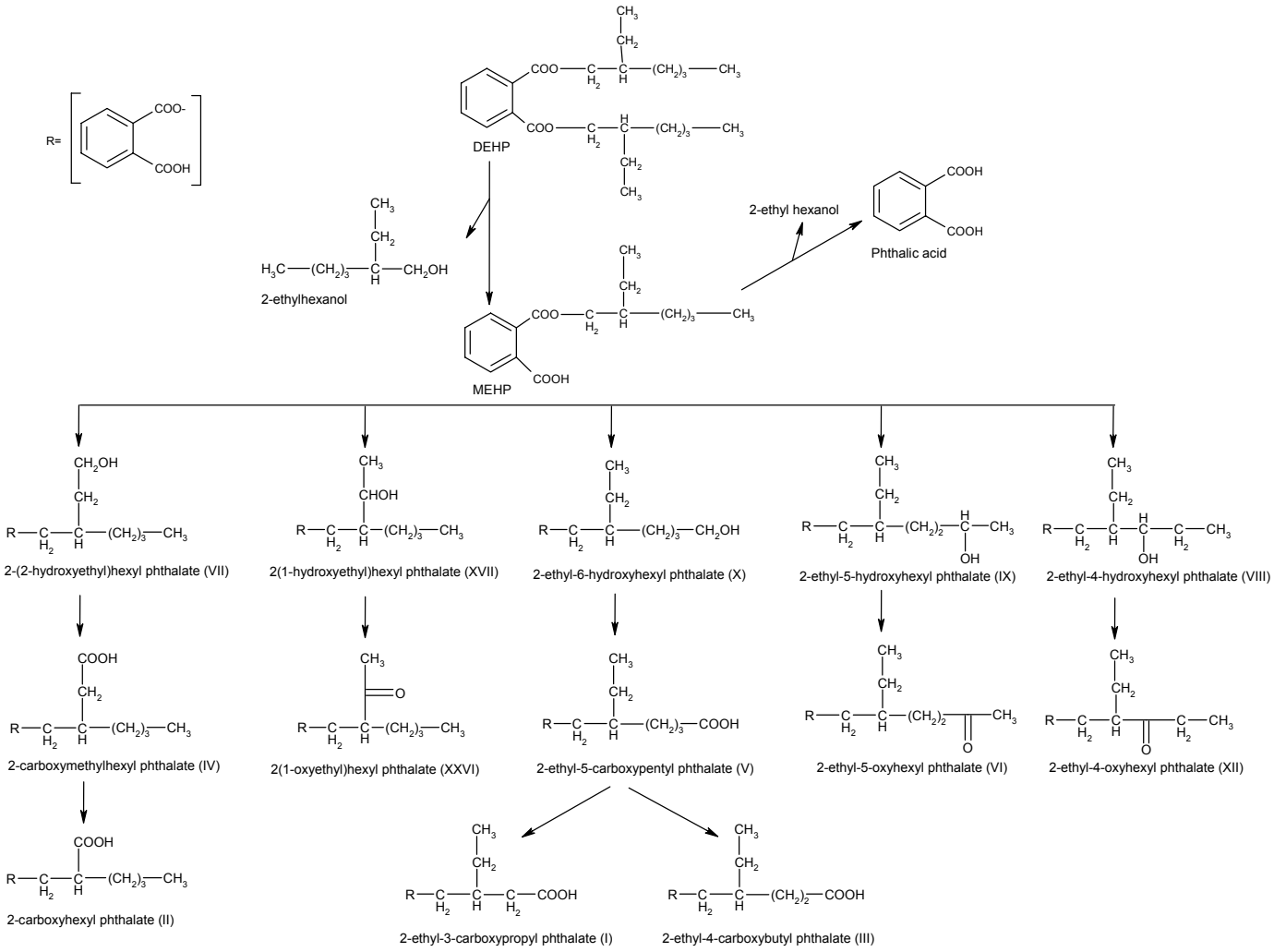
Animal data generally support the human findings. DEHP is hydrolyzed in the small intestines and absorbed as MEHP and 2-ethylhexanol. At high concentrations, a limited amount of unhydrolyzed DEHP might be absorbed. The degree of gastrointestinal absorption varies among animal species and is apparently greater in rodents than in monkeys. Animal studies indicate that DEHP might be absorbed through the skin in minute quantities. Absorption via the respiratory tract has also been indicated, although quantitative absorption studies have not been published.

Limited human data from autopsies have indicated the presence of DEHP in adipose tissues and kidneys.

Metabolic pathways for DEHP involve a number of reactions, as presented in Figure 3-4. Esteratic or hydrolytic cleavage of DEHP results in the formation of MEHP and 2-ethylhexanol. The esterases responsible for these hydrolytic steps are found in numerous body tissues, but highest levels occur in the pancreas (hydrolytic reactions occur more readily following oral exposure because of the high content of esteratic activity within the gastrointestinal tract). MEHP is further metabolized via numerous oxidative reactions, resulting in the formation of 30 or more metabolites, some of which can be conjugated with glucuronic acid for excretion. Oxidation of 2-ethylhexanol primarily yields 2-ethylhexanoic acid and several keto acid derivatives, which are excreted in the urine.

In orally-exposed humans, approximately 65% of DEHP metabolites are excreted in the urine as glucuronide conjugates. The aglycone moiety of these conjugates as well as the nonconjugated DEHP metabolites excreted by humans are similar to those found in urine and feces of laboratory animals, although relative proportions might differ with species, dose, and time. No studies were located regarding fecal excretion of DEHP metabolites in humans. However, significant amounts of DEHP were noted in the feces of animals given DEHP by the oral route; it presumably represents unmetabolized

Figure 3-4. DEHP Metabolites[a]



[a]The metabolites illustrated in the metabolic pathway outlined above are oxidized at only one carbon site. Additional metabolites which are oxidized at two carbons sites are not shown in this figure.

Adapted from Albro 1986

DEHP. MEHP and other metabolites were frequently found in feces of DEHP-exposed animals, in some cases associated with biliary excretion products.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

No quantitative data regarding absorption after inhalation exposures of humans or animals to DEHP were located. However, absorption can occur through the lungs of humans as evidenced by identification of DEHP in the urine or lung tissue of infants exposed to DEHP during respiration therapy (Roth et al. 1988).

In rats, inhalation of an aerosol containing 1,000 mg/m^3 of DEHP resulted in peroxisome proliferation (Merkle et al. 1988), indicating that absorption had occurred. However, no quantitative details were provided which could be used to estimate absorption in this study, or rule out inadvertent co-exposure by the oral route.

3.4.1.2 Oral Exposure

Measurement of DEHP excretory metabolites indicate that about 11–15% of a 30 mg oral dose is excreted in human urine and, therefore, was absorbed (Schmid and Schlatter 1985). However, the total absorption is probably higher (perhaps 20–25%), since animal studies indicate that biliary excretion accounts for 15–20% of the absorbed dose (see Section 3.4.4.3).

Analysis of animal data suggests that rodents absorb DEHP better than other animal species (Astill 1989; Rhodes et al. 1986). DEHP is absorbed primarily as MEHP and 2-ethylhexanol, along with small amounts of unhydrolyzed DEHP. At low concentrations, most of DEHP is hydrolyzed in the small intestines and absorbed as the MEHP and 2-ethylhexanol (Albro 1986; Albro et al. 1982b). At high concentrations, some unhydrolyzed DEHP is also absorbed. Based on urinary excretion of metabolites, rats can absorb at least 55% of a 2,000 mg/kg oral dose of DEHP (Rhodes et al. 1986). Actual absorption is probably greater than this since biliary excretion of previously absorbed and metabolized DEHP might account for a considerable portion of the fecal elimination. Larger percentages of smaller doses will be absorbed, since intestinal transport of MEHP and DEHP can be saturated at high doses (Short et al. 1987).

A similar saturation phenomenon is seen in monkeys, although monkeys appear to absorb a smaller percentage of each oral dose than do rats (Astill 1989; Rhodes et al. 1986).

3.4.1.3 Dermal Exposure

DEHP does not appear to be readily absorbed through the human skin. Wester et al. (1998) estimated that dermal absorption amounts to approximately 1.8% of a 24-hour applied dose of DEHP solubilized in ethanol. They noted that 1.1% of the radioactivity from a 24-hour dermal application of ^{14}C-labeled DEHP to the forearm of volunteers was excreted in the urine within 7 days postapplication. They used this finding and the observation that approximately 60.8% of an intravenously-injected dose of DEHP was excreted in the urine of Rhesus monkeys over the same posttreatment time period as the basis for their estimate. No other reports were located regarding dermal absorption of DEHP in humans.

DEHP has been shown to be poorly absorbed through the skin of laboratory animals. Dermal absorption was reported to be approximately 5% in rats exposed for 7 days to an initial dose of 30 mg DEHP/kg (dissolved in ethanol); about 95% of the original radioactivity was recovered from the application site and 5% in the urine and feces (Melnick et al. 1987). In more recent investigations concerning the migration of DEHP from PVC film and subsequent dermal absorption, Deisinger et al. (1998) subjected rats to 24-hour dermal (rats were sacrificed after the 24-hour application period) applications of radiolabeled DEHP (approximate dose 1,739 mg DEHP/kg) contained in occluded PVC film patches, and estimated that approximately 0.0045% of the applied dose was dermally absorbed during the exposure period after accounting for radioactivity recovered in the PVC film, urine, feces, cage washes, and carcass. The mean calculated dermal absorption rate was 0.242 $\mu g/cm^2$/hour. Similarly exposed rats whose PVC was removed after the 24-hour exposure and were followed for 6 days postexposure exhibited a similar calculated absorption rate (0.239 $\mu g/cm^2$/hour), but had a slightly higher percent total absorption (0.01%). *In vitro* studies of DEHP absorption through human, rat, and porcine epidermal segments confirm the poor dermal absorption of DEHP (Scott et al. 1987; Wester et al. 1998). The steady state dermal absorption rate was only 1.06 $\mu g/cm^2$/hour in human skin segments and 2.24 $\mu g/cm^2$/hour in rats; approximately 3.9% of an applied dose of DEHP penetrated perfused porcine skin flaps. *In vivo* absorption of DEHP from polyvinyl chloride film by shaved rats and *in vitro* data on rat and human dermal absorption rates were used to estimate an absorption rate from PVC film for humans of 0.016 $\mu g/cm^2$/hour (Deisinger et al. 1991).

3.4.2 Distribution

Few studies were located regarding the distribution of DEHP in humans after exposure to DEHP. DEHP is lipophilic and tends to accumulate in adipose tissue. DEHP was present in human adipose tissues from accident victims at a concentration of 0.3–1.0 ppm (Mes et al. 1974) and in 48% of the adipose tissue specimens from cadavers autopsied in 1982 as part of the Human Adipose Tissue Survey from the National Human Monitoring Program (EPA 1989b). In addition, DEHP has been isolated in the kidneys of autopsied patients (Overturf et al. 1979). The presence of DEHP in tissues might be an artifact since DEHP can easily contaminate biological samples during laboratory processing operations. This can, at times, make DEHP appear to be more ubiquitous in tissues than it actually is.

As detailed in Section 3.2.2.6 Developmental Effects, DEHP and/or metabolites administered during gestation can induce increased incidences of resorptions, fetal deaths and malformations in rats and mice (Hellwig et al. 1997; Ritter et al. 1987; Shiota and Mima 1985; Tomita et al. 1982a; Tyl et al. 1988; Yagi et al. 1980). While this might be interpreted as evidence of transplacental transfer of these substances, the possibility that the effects result from toxicity to the dams cannot be ruled out.

3.4.2.1 Inhalation Exposure

No studies were located regarding the distribution of DEHP in the tissues of humans or animals after inhalation exposure to DEHP.

3.4.2.2 Oral Exposure

No studies were located regarding the distribution of DEHP in humans after oral exposure to DEHP. However, since environmental exposures to DEHP occur primarily through foods, the data discussed in Section 3.4.2 concerning the presence of DEHP in human adipose deposits is a reflection of distribution following oral exposures, assuming that contamination was avoided.

The liver, kidney, testes, and blood were identified as sites of DEHP metabolism or utilization after 14-day oral exposure of rats to a 2,000 mg/kg/day dose containing ^{14}C-DEHP labeled in the phenyl ring (Rhodes et al. 1986). At the end of the 14 days, the liver contained 205 µg/g, the kidney 105 µg/g, the blood 60 µg/g, and the testes 40 µg/g of DEHP equivalent. A very similar distribution pattern was seen in monkeys under the same exposure conditions (2,000 mg/kg/day), although the actual tissue

concentrations were 10–15% of the amounts in the rat, reflecting the lower coefficient of absorption for DEHP in the monkey and possible differences in other aspects of pharmacokinetics (Rhodes et al. 1986). The tissue distribution in the monkeys, 7 days after administration of a single dose of 2,000 mg/kg DEHP, was similar to that observed after continuous exposures, except that the concentration of the label in the testes (3.75 µg/g) was greater than that in the liver and kidney (2.5 µg/g). The concentration of the label in the blood was <50% of that in the liver and kidney.

The tissue distribution of a single radiolabeled dose of DEHP was investigated in rats, dogs, and miniature pigs given 50 mg/kg/day DEHP in the diet for 21–28 days before receiving the radiolabeled dose (5 µCi/kg in 50 mg/kg) by gavage or gelatin capsule (Ikeda et al. 1980). Tissue samples were evaluated in each species at 4 hours, 24 hours, and 4 days after administration of the radiolabeled sample.

In all three species, the liver contained a substantial portion of the tissue label at 4 and 24 hours (Ikeda et al. 1980). Label was cleared from the dog liver less rapidly than from the liver of rats and pigs. Dogs also contained a relatively high percentage of label in muscle tissue, approximately twice that found in pigs. (The presence of label was not determined in rat muscle). In dogs, there was more of the label in the muscle at 4, 24, and 96 hours than in the fat tissue. In pigs, there was much more label in the fat than in the muscle. In all cases the amount of label in the tissues examined was <2% of the administered dose, except for a value of 2.24% in the rat liver.

DEHP demonstrated a lower affinity for brain tissue than liver in mice following oral administration of 0.7 mg DEHP/kg to 3-, 10-, and 20-day-old animals (Eriksson and Darnerud 1985). The retention of the label in the brain was minimal, but was greater in 3-day-old mice than in older mice. In 3-day-old mice, 1.6% of the label was identified in the brain 24 hours after administration as opposed to approximately 0.25% in 10-day-old mice and values below detection for the 20-day-old mice. This might indicate increased permeability of the blood-brain barrier to DEHP in neonates or developing organisms and help to explain the finding that there is an increase in neuronal peroxisomes in newborn mice exposed to DEHP (Dabholkar 1988). Alternatively, the higher level of DEHP in the brain might be a reflection of a slower rate of compound elimination in neonates.

3.4.2.3 Dermal Exposure

No studies were located regarding the distribution of DEHP in human tissues after dermal exposures to DEHP. In rats exposed to 30 mg/kg DEHP applied to the skin for 7 days, the liver contained 0.063% of

the applied dose, the kidney 0.012%, the muscles 1.162%, and the fat 0.066% (Elsisi et al. 1989; Melnick et al. 1987). The small intestines were found to contain 0.161% of the dose, giving evidence for either intestinal uptake of DEHP from non-oral routes of exposure or the excretion of metabolites in the bile.

3.4.2.4 Other Routes of Exposure

Humans can be exposed to DEHP through medical practices such as dialysis, respiration therapy, blood transfusions, or total parenteral nutrition treatment (TPN) where the source of DEHP is the plastics utilized in medical treatment devices or storage bags. Thus, data on tissue distribution following alternate routes of exposure are important in evaluating the potential for DEHP to have an adverse effect on human health. Exposure can occur through the intravenous route as well as the oral, dermal, and respiratory routes. In addition, limited formation of MEHP occurs in storage bags, so that exposure includes DEHP plus MEHP (FDA 2001h).

DEHP exposures through the parenteral route bypass the intestinal esterases so there will tend to be a greater proportion of the dose entering systemic circulation as DEHP rather than MEHP. This is evident in data from human studies following exchange transfusions and hemodialysis. Initially there is more DEHP than MEHP in the blood (Pollack et al. 1985a; Sjoberg et al. 1985d). However, DEHP levels decline rapidly with a half-life of 10 hours (Sjoberg et al. 1985d), and the MEHP levels increase until the time-averaged concentrations are roughly equal (Pollack et al. 1985a). It was not determined whether the differences in disappearance were due to volume of distribution or metabolism differences. Between exposures, both DEHP and MEHP disappear from the blood, and phthalic acid concentrations increase. Blood phthalic acid concentrations correlate better with the duration of dialysis than either DEHP or MEHP levels (Pollack et al. 1985a). Similar results were seen in animal studies. After arterial injection, DEHP was rapidly cleared from the blood of rats (Pollack et al. 1985b).

The distribution of parenterally administered DEHP to soft tissues was studied in marmoset monkeys. Seven days after intravenous and intraperitoneal administration of 100 and 1,000 mg/kg doses in corn oil emulsion, respectively, the lungs were the major tissue retaining DEHP label (Rhodes et al. 1986). Following intravenous administration, the concentration of DEHP in the lungs was 4,257 µg/g; that in the liver, approximately 17 µg/g; the testes, 5 µg/g; and the kidney, 4 µg/g. The authors hypothesized that the DEHP in the lungs reflects entrapment of insoluble compound from the intravenous emulsion by alveolar capillaries. After intraperitoneal administration, the concentrations of label in the lung, liver, kidney, and testes were approximately 265, 80, 60, and 15 µg/g, respectively. Seven days after

intraperitoneal administration, 85% of the dose remained in the peritoneal cavity. Although the results of the intravenous and intraperitoneal distribution experiments appear to indicate that DEHP can deposit in the lungs following parenteral exposures, there is insufficient basis to conclude this because only total radioactivity was measured, and DEHP might have been sequestered in the capillaries of the lungs due to a mechanical artifact of the solubilization rather than by being absorbed by the pulmonary parenchyma. Accumulation of DEHP in the lungs does not occur with oral or dermal exposures and is not expected to occur after inhalation exposure, although none of the studies conducted using this route have evaluated concentrations of DEHP in the tissues.

3.4.3 Metabolism

Based on data from both human and animal studies, the metabolism of DEHP involves a complex series of reactions with the production of 30 or more metabolites (Albro 1986; Albro et al. 1982a, 1982b, 1983, 1987; Astill 1989; Schmid and Schlatter 1985). The first step in metabolism is the hydrolytic cleavage of DEHP resulting in the formation of MEHP and 2-ethylhexanol. This step occurs rapidly in the intestine, as evidenced by the disappearance of DEHP from rat gut homogenate (half-life 12.6 minutes), and the formation of approximately equal proportions of MEHP and 2-ethylhexanol (Barber et al. 1994). The formation of MEHP from the parent ester is acheived by lipases that have been identified in the pancreas and intestinal mucosa, as well as the liver, kidneys, lungs, skin, and plasma. The pancreatic tissue is the richest source of these esterases by several orders of magnitude (Albro 1986). The adipose tissue has the lowest concentration of esterase. Because of differences in tissue enzyme activities, DEHP exposure by the oral route results in a larger portion of the dose being converted to MEHP and 2-ethylhexanol than occurs with inhalation or dermal exposures. Hydrolysis of the second ester bond converts a small portion of the MEHP to phthalic acid but most of the MEHP undergoes ω- (omega) and ω-1-oxidation of the aliphatic side chain. The ω-oxidation step can be followed by α- or β-oxidation reducing the number of carbons in the 2-ethylhexyl side chain. A proposed metabolic pathway for oxidation of MEHP is presented in Figure 3-4. Each of the metabolites depicted in Figure 3-4 has been given a Roman numeral designation which is often used in place of its chemical name (Albro et al. 1983). After the primary metabolic conversions described above, the oxidized derivatives of MEHP can be conjugated with glucuronic acid for excretion (Albro 1986; Astill 1989). There is no evidence to suggest that the aromatic phthalic acid moiety is degraded during the metabolism of DEHP.

2-Ethylhexanol is also metabolized through oxidative pathways. The primary urinary products from 2-ethylhexanol are 2-ethylhexanoic acid and several keto acid derivatives which appear to be products of β-oxidation (Albro and Corbett 1978).

No data were located regarding the metabolites produced in humans or animals after either inhalation or dermal exposures to DEHP. Metabolism following these routes of exposure is expected to be similar to that after oral exposures, since there are lipases present in the alveolar cells of the lungs and the epidermis. However, the activities of these lipases are about 20% of that for the pancreatic esterase secreted into the intestines (Albro et al. 1987), so it is possible that a larger portion of an absorbed dose from respiratory or dermal exposures to DEHP will initially be presented to the tissues as unhydrolyzed DEHP rather than MEHP.

There have been studies of the metabolism of DEHP in humans after oral exposures as reflected by its urinary excretory products. In two volunteers exposed to 30 mg DEHP, metabolites I, II, III, IV, V, VI, VII, and VIII were identified in the urine by mass spectroscopy (Schmid and Schlatter 1985). MEHP accounted for 6–12% of the measured metabolites. Metabolite VI was approximately 20% of the excreted material, Metabolite IX approximately 30% and Metabolite V approximately 30%. The remaining metabolites were each less than 5% of the excreted material. Based on a comparison of the metabolites in the hydrolyzed urine as compared to the unhydrolyzed urine, approximately 65% of DEHP metabolites are excreted as glucuronide conjugates in humans. Each of these major metabolites is the product of oxidation of a different carbon in the 2-ethylhexyl substituent.

The same DEHP metabolites are found in the urine and feces of monkeys, rats, mice, guinea pigs, and hamsters, although there are some differences in the proportion of the metabolites excreted as conjugates of glucuronic acid (Albro et al. 1982a; Astill 1989). Monkeys are similar to humans and excrete 60% of the urinary metabolites as conjugates (Albro et al. 1982a). Guinea pigs, hamsters, and mice appear to excrete a smaller and more variable portion of DEHP metabolites as conjugates, while none of the metabolites in rat urine are found as conjugates (Albro et al. 1982a). Based on examination of the urinary metabolites after hydrolysis with aryl sulfatase, acylase I, and carboxypeptidase A, there is no evidence that conjugation with glutathione, sulfates, or amino acids occurs in rats, mice, guinea pigs, or hamsters (Albro et al. 1982a). The quantities of free DEHP excreted are minimal in all species that have been examined. The amount of MEHP varies from species to species. A large fraction (72%) of MEHP was excreted in the urine of guinea pigs (Albro et al. 1982a). Monkeys and mice excreted 17–18% MEHP (Albro et al. 1982a; Rhodes et al. 1986); hamsters, 4.5% MEHP; and rats only traces of this compound

(Albro 1986; Albro et al. 1982a; Astill 1989). Mice and hamsters excrete more phthalic acid than rats and guinea pigs (Albro 1986; Albro et al. 1982a).

Metabolites I, IV, V, VI, VIII, and IX were the metabolites present in the highest concentrations in the animal species studied (Albro et al. 1982a, 1982b, 1987; Astill 1989; Rhodes et al. 1986; Short et al. 1987). The relative proportions of metabolites differ with species, dose, and time (Lhuguenot et al. 1985, 1988). The metabolites with multiple side chain oxidation sites are more water soluble than those with a single hydroxyl, carbonyl, or carboxyl functional group. The multiple oxidation site metabolites (XII through XXV) have, accordingly, been more difficult to isolate and quantify (Albro et al. 1983).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

No data were located concerning excretion in humans or animals after inhalation exposures to DEHP.

3.4.4.2 Oral Exposure

Humans exposed to a single dose of 30 mg DEHP excreted 11 to 15% of the dose as metabolites in the urine over 48 hours (Schmid and Schlatter 1985). When a smaller dose (10 mg) was given for each of 4 sequential days, 15–25% of the dose was recovered in the urine. No measurements of fecal elimination of DEHP or its metabolites were made.

The data from animal studies demonstrate that DEHP and it metabolites are excreted in both the urine and the feces. Rats exposed to 50–300 mg/kg DEHP excrete 32–70% of the dose in the urine as metabolites (Astill 1989; Ikeda et al. 1980; Short et al. 1987). An additional 20–25% of the absorbed dose was excreted with the bile in the fecal matter. The remainder of the fecal excretion was unabsorbed DEHP and MEHP. In monkeys, approximately 30% of a 100 mg/kg dose and 4% of a 2,000 mg/kg dose were excreted in the urine (Astill 1989; Rhodes et al. 1986; Short et al. 1987; Sjoberg et al. 1985b). The remainder was in the feces. A portion of the fecal metabolites was contributed by the bile. The biliary excretory products represent approximately 15% of the absorbed DEHP. In rats and mice administered radiolabeled DEHP, 85–90% of the label was excreted in the first 24 hours (Astill 1989; Ikeda et al. 1980). In monkeys, a smaller portion of the label (50–80%) was excreted in the first 24 hours (Astill 1989). Twenty-four hour excretion of label in urine and feces was also lower in dogs (67%) and

miniature pigs (37%) than in rats and mice (Ikeda et al. 1980). Miniature pigs were unique in that very little label (26%) was found in the feces even with 4 day collected samples. This compares with values of 53% in rats and 75% in dogs 4 days after administration of a 50 mg/kg dose containing 5 µCi/kg of radiolabel. It appears that biliary excretion of metabolites is very limited in miniature pigs.

Because of their lipophilic nature, both DEHP and MEHP can accumulate in breast milk and subsequently be transferred to suckling offspring. For example, Dostal et al. (1987b) detected both DEHP (216 µg/mL) and MEHP (25 µg/mL) in the milk collected from lactating Sprague-Dawley rats 6 hours after the last of three daily gavage doses of 2,000 mg DEHP/kg (Dostal et al. 1987b). At this time, no DEHP could be detected in dams' plasma, but substantial amounts of MEHP were detected. Pups' plasma had no detectable DEHP or MEHP. Cimini et al. (1994) and Stefanini et al. (1995) also presented evidence of transfer of DEHP and/or metabolites to offspring via breast milk. In all of these studies, there was increased peroxisomal enzyme activities in pups' livers.

3.4.4.3 Dermal Exposure

A mean of 1.1% of the radioactivity from 24-hour dermal application of radiolabeled DEHP was recovered in the urine of six volunteers 7 days postapplication sampling (Wester et al. 1998).

DEHP derivatives were excreted in both the urine and feces following dermal exposures of rats to 30 mg/kg for 7 days (Elsisi et al. 1989). Only 5% of the dose was excreted; 3% was in the urine and 2% in the feces. After 7 days, 95% of the dose was recovered from the skin surface. The finding of approximately 40% of the excreted label in the fecal matter is a reflection of the importance of the biliary excretion route.

3.4.4.4 Other Routes of Exposure

Twenty-four hours after a single intravenous injection of ^{14}C-DEHP to rats, the radioactivity was mainly recovered in the urine and feces, suggesting that the major excretory pathways in rats are the urine and bile (Schulz and Rubin 1973). Excretion was dose-dependent as shown by the fact that 50–60% of the injected radioactivity from a low dose was recovered in urine and feces, whereas less than 50% was recovered when a high dose was injected (Schulz and Rubin 1973). In Rhesus monkeys, 60.8% of the radioactivity in an intravenously injected dose of radiolabeled DEHP was excreted in the urine during 7 days postinjection, more than half of which was eliminated in the first day (Wester et al. 1998).

Medical exposure to DEHP routinely occurs during intravenous infusion of blood and blood products (e.g., fluids and medication). Rapid elimination of DEHP that reaches the blood during transfusions and hemodialysis has been demonstrated in several studies (Barry et al. 1989; Lewis et al. 1978; Rubin and Schiffer et al. 1975; Sjorberg et al. 1985). For example, transfusion of platelets that were stored in vinyl plastic packs resulted in blood levels of DEHP (peak plasma concentrations ranging from 0.34 to 0.83 mg/dL) that fell monoexponentially with a mean rate of 2.83% per minute and a half-life of 28 minutes (Rubin and Schiffer 1975). Similarly, measurements on patients who had undergone >50 hemodialysis treatments indicated that most of the DEHP present in the serum at the completion of a dialysis session is likely to be gone in 5–7 hours (Lewis et al. 1978).

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

Physiologically based pharmacokinetic (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 end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

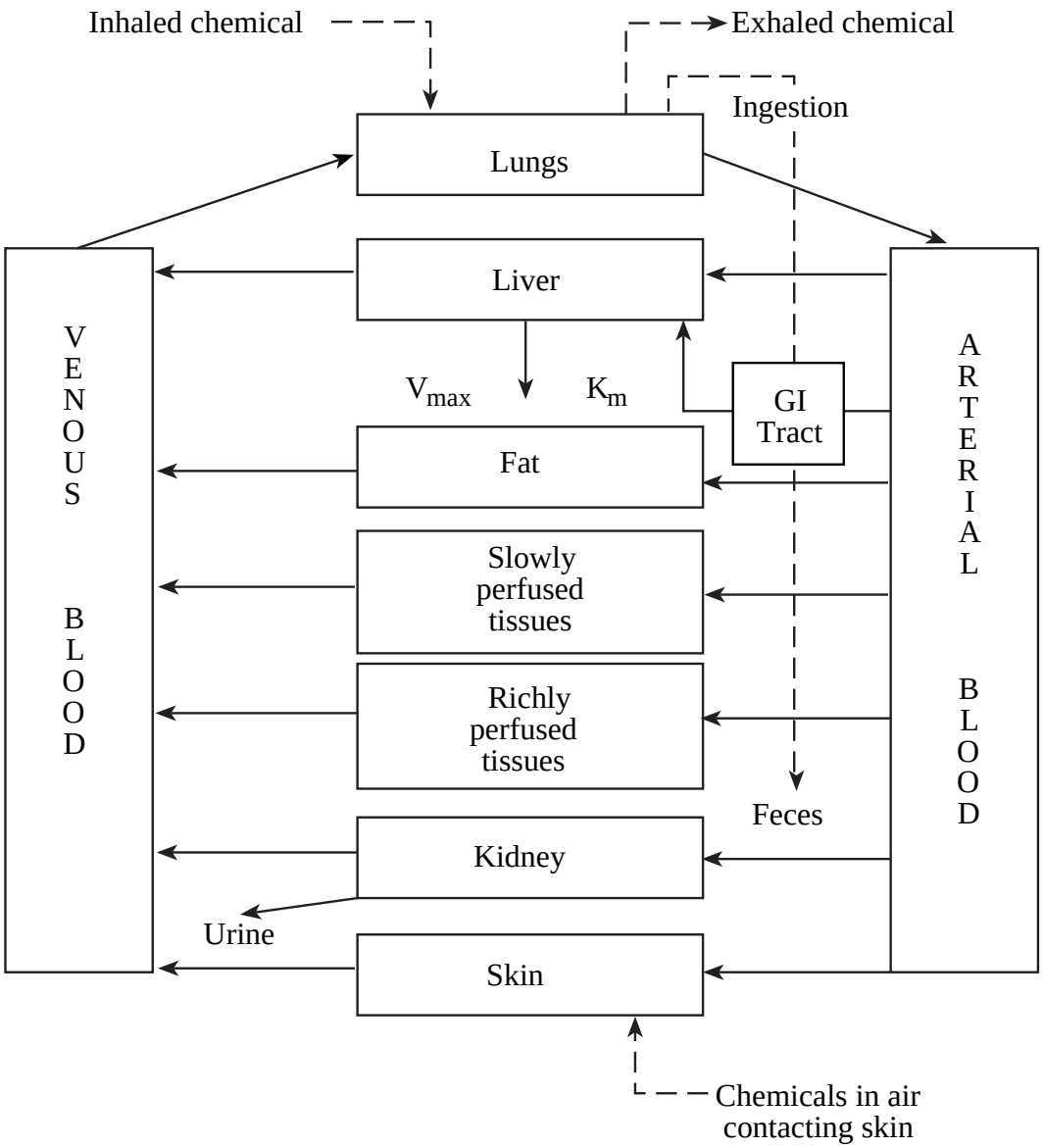
The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen

1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-5 shows a conceptualized representation of a PBPK model.

Figure 3-5. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Keys et al. (1999) describe a PBPK model of DEHP in rats that simulates the pharmacokinetics of both DEHP and its major metabolite, MEHP. The model is intended for use in simulating doses of MEHP to the testes resulting from oral exposures to DEHP.

Description of the Model. The Keys et al. (1999) model simulates six tissue compartments: small intestine, blood, liver, testis, slowly perfused tissues, and poorly perfused tissues. Conversion of DEHP to MEHP in the small intestine is simulated with k_m and V_{max} terms, whereas conversion in liver and blood are simulated with separate first order rate constants, k_l and k_b, respectively. Elimination of DEHP and MEHP is assumed to be entirely by metabolism of DEHP to MEHP, and MEHP to unspecified metabolites; the latter transformation is represented in the model by k_m and V_{max} terms.

Keys et al. (1999) explored four approaches to modeling the pharmacokinetics of DEHP and MEHP. In a flow-limited version of the model, transfers between blood and tissues are simulated as functions of blood flow, tissue concentrations of DEHP or MEHP, and tissue:blood partition coefficients, assuming instantaneous partitioning of the compounds between tissue and blood (Ramsey and Anderson 1984). In a diffusion-limited version of the model, the tissue transfers include a first order rate term (referred to as the permeation constant) that relates the intracellular-to-extracellular concentration gradient to the rates of transfer. This model required estimates of extracellular tissue volume (ECV) and intracellular volume (ICV); ECV is assumed to be equal to tissue blood volume and ICV is assumed to be equal to the difference between tissue blood volume and total tissue volume. This approach would be expected to underestimate the true ECV of most tissues, which is approximately 45% of tissue mass (Edelman and Leibman 1959), and overestimate the true ICF; the significance of these potential differences are not discussed by Keys et al. (1999). In a pH-trapping version of the model, instantaneous partitioning (i.e., diffusion-limited) of only the nonionized species of MEHP between the intracellular and extracellular compartments of tissues is assumed, and the respective concentrations of the non-ionized and ionized species in each compartment are predicted by the pK_a for the carboxylic acid moiety of MEHP (pK_a is assumed to be 3.76, and intracellular pH is assumed to be approximately 7–7.05, depending on the tissue). A diffusion-limited version of the model is also described that includes a simulation of enterohepatic circulation of MEHP, in which the transfer of MEHP from the liver to the small intestine is represented with a first order rate constant (diffusion-limited) and a time delay constant for the subsequent reabsorption of MEHP from the small intestine.

Tissue:blood partition coefficients for DEHP and nonionized MEHP were estimated from their n-octanol:water partition coefficients (K_{ow}), using the approach reported by Poulin and Krishnan (1993).

Tissue:blood partition coefficients for total MEHP (ionized and nonionized) were determined experimentally using a vial-equilibration method with correction for pH (Table 3-5).

The models were developed to simulate the physiology (e.g., blood flows and body composition) of adult rats (Table 3-6). These parameter values were then extrapolated to juvenile rats to accommodate calibration and validation data in which juvenile rats were the test organisms. The extrapolation was achieved by scaling blood flows, metabolic constants, and adipose volumes to various functions of body weight (e.g., allometric scaling).

Keys et al. (1999) note that certain model parameter values were estimated by applying a step-wise parameter optimization routine to data on blood or tissue levels following oral or intravenous exposure to DEHP and MEHP. The parameters estimated included the k_m and V_{max} values for metabolism of DEHP and MEHP, and first order rate constants for the following parameters: metabolism of DEHP (e.g., liver), absorption of DEHP and MEHP in the small intestine, intracellular-to-extracellular transfer of nonionized MEHP, and biliary transfer of MEHP from liver to small intestine (these values are not provided in the profile because they are derived from optimization procedures and might not be directly useful for other models). Keys et al. (1999) do not explicitly cite or describe the data sets used to optimize model parameter values, or distinguish the data used in optimization from data used in validation exercises. Based on Table 5 of their report, it appears that at least some data from Pollack et al. (1985b) were used to optimize the model.

Validation of the Model. Output from the various models were compared to observations of blood and testes concentrations reported from studies of oral gavage or intravenous exposures of rats to DEHP or MEHP (Oishi 1989a, 1990; Pollack et al. 1985b; Teirlynck and Belpaire 1985). Based on the comparisons of model outputs to observed time courses for blood MEHP concentrations from Pollack et al. (1985b), Keys et al. (1999) conclude that the pH-trapping model more closely represents the empirical data. However, it is difficult to interpret this finding if the same data were used in the model optimization (see Table 5 of Keys et al. 1999). The pH-trapping model simulated reasonably well the time courses for blood and testes concentrations of MEHP reported by Oishi (1989a, 1990) and Teirlynck and Belpaire (1985); however, comparisons to the output of other versions of the model are not presented.

Table 3-5. Tissue:Blood Partition Coefficients Used in the Keys et al. (1999) Model

Tissue	DEHP (estimated)	Non-ionized MEHP (estimated)[a]	Total MEHP (experimental)[b]	Total MEHP (estimated)
Liver	21.8	21.6	1.70±32	3.0
Fat	351.0	351.0	0.12±0.05	44.2
Muscle	6.1	6.0	0.38±0.23	1.5
Testes	6.5	6.5	1.02±0.07	1.6

[a]from K_{ow} based on algorithms from Poulin and Krishnan (1993)
[b]vial equilibration study with pH correction

Table 3-6. Physiological Parameter Values Used in the Keys et al. (1999) Model

Description	Value
Body weight (kg)	0.89–0.38
Cardiac output (L/hour-kg$^{0.75}$)	15.33
Blood flow as a fraction of cardiac output:	
Liver	0.183
Fat	0.07
Testes	0.013
Slowly perfused tissues	0.157
Rapidly perfused tissues	0.577
Compartment volumes as fraction of body weight:	
Liver	0.0366
Fat	0.034–0.08
Testes	0.01
Blood	0.07
Small intestine	0.0139
Slowly perfused tissues	0.73–0.78
Rapidly perfused tissues	0.0534
Compartment blood volume as fraction of tissue volume[a]:	
Liver	0.21
Fat	0.05
Testes	0.03
Slowly perfused tissues	0.04
Rapidly perfused tissues	0.21

[a]This volume was assumed to represent tissue extracellular volume.

Risk Assessment. The model provides an approach to estimating doses of MEHP to the testes of the rat following oral doses of DEHP and might be useful for internal dose-response assessment of rat bioassay data in which the toxicity end point of interest is testicular toxicity. However, such uses of the model, or other potential uses in risk assessment, have not been evaluated.

Target Tissues. Output from the model, for which validation exercises were conducted, are predictions of blood and testes concentrations of MEHP.

Species Extrapolation. The model is designed to predict the blood and testes concentrations of MEHP following oral doses of DEHP to rats. Extrapolation to other species would require modification of the model to account for different tissue masses, blood flows, and possibly other kinetic variables.

Interroute Extrapolation. The model is designed to simulate the pharmacokinetics of DEHP and its metabolite, MEHP, when exposure is by the oral route. The pharmacokinetics of DEHP would be expected to be different for other routes of exposure; therefore, the output of the model cannot be extrapolated to other routes (e.g., dermal, inhalation) without modification of the model. Calibration and validation studies utilized gavage exposures for oral dosing, and therefore, the model might not be applicable to other oral exposure pathways (e.g., dietary, drinking water) without modification.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

DEHP is hydrolyzed to the monoester, MEHP, in the gastrointestinal tract by a pancreatic lipase. Hydrolytic activity varies considerably between species, the highest being in the mouse, followed by the rat, guinea pig, and hamster (Albro 1986; Lake et al. 1984a). In humans and primates, hydrolysis is considerably slower than in rats (Albro et al. 1982a; Rhodes et al. 1986). Of DEHP and MEHP, the former is considerably more lipophilic, and would be expected to be more readily absorbed in the gastrointestinal tract. However, using an everted gut-sac preparation from the rat small intestine, White et al. (1980) observed that all of the DEHP that entered the serosal buffer was in the monoester form. These findings suggested the presence of a hydrophilic barrier that limits the amount of DEHP that is absorbed in the intestines. This means that absorption is not only species-dependent but also dose-dependent, as shown in several *in vivo* studies (Albro et al. 1982a; Astill 1989; Rhodes et al. 1986). Furthermore, there also appears to be age-related differences in absorption as shown by Sjoberg et al. (1986a, 1986b) in rats

administered DEHP by gavage; absorption was greater in young animals. Sjoberg et al. (1985b) suggested that the relatively higher proportion of intestinal tissue in relation to body weight and the relatively higher blood flow through the gastrointestinal tract might be factors causing increased absorption in young animals. The mechanism of dermal absorption of DEHP is not known.

Experiments have been conducted to simulate leaching of phthalates from blood bags by allowing human plasma to extract added phthalates from coated Celite (Albro and Corbett 1978). It was found that more than 80% of the DEHP was associated with lipoproteins, in the order LDL > VLDL > HDL > chilomicrons. The remaining DEHP was adsorbed weakly and nonspecifically to other proteins including albumin. MEHP was in equilibrium between free in solution and adsorbed to albumin, no MEHP was bound to lipoproteins. Rock et al. (1986) reported that in human plasma, the lipase that hydrolyzes DEHP copurified with the albumin, and once in the plasma, the MEHP bound to albumin. An earlier study by Jaeger and Rubin (1972) reported that in human blood stored in PVC bags, the bulk of DEHP was associated with lipoproteins, but a substantial amount was in a fraction likely to represent DEHP soluble in plasma water as well as bound to plasma proteins and cell membranes.

Oral administration of DEHP to rats always results in higher levels of MEHP than DEHP in blood, but after intravenous dosing, DEHP predominates because first pass conversion to MEHP (i.e., the putative toxicant) is avoided. Intravenous exposure from medical procedures therefore might have a lower potential for toxicity compared to oral exposure, even though high level human exposure is not expected to occur from oral exposure, but rather from intravenous exposure. Studies by Sjoberg and coworkers showed that after gavage dosing, DEHP levels in blood were 40–50% of the MEHP levels and 35–70% after intravenous injection (Sjoberg et al. 1985a, 1986a, 1986b). In general, after oral dosing, there is only a weak correlation between the dose applied and the resulting blood level (Huber et al. 1996). This might be due to different dosing regimes, time of blood sampling after dosing, and failure to consider the capacity of erythrocytes to store DEHP (Huber et al. 1996).

3.5.2 Mechanisms of Toxicity

Mechanisms for the major effects of DEHP are discussed in this section. In animals, these effects include hepatotoxicity and alterations of the male reproductive system. In rodents, particularly rats and mice, hepatotoxicity leads to liver cancer if exposure is sustained; therefore, the mechanisms of liver effects and cancer are discussed together. An exhaustive discussion on mechanisms of action is beyond the scope of this document. Rather, the information below is intended only as a summary of the most important

issues. Although information is available on the underlying biological mechanisms of DEHP in animals, demonstration of these mechanisms does not necessarily mean that exposure will cause health effects in humans.

Numerous comprehensive reviews on the role of peroxisome proliferation in liver cancer have been published in recent years and much of the information in summarized in this section has been gleaned from them (Cattley and Roberts 2000; Cattley et al. 1998; Doull et al. 1999; Green 1995; Huber et al. 1996; Lake 1995; Melnick 2001; Rao and Reddy 1996; Tugwood et al. 1996). As discussed below and in Section 3.5.3, humans are nonresponsive to peroxisomal proliferation and are probably less susceptible to liver cancer than rodents due to the species specificity of the mechanism. DEHP is best classified as a nongenotoxic epigenetic chemical that can reversibly inhibit gap junctional intercellular communication and thereby alter homeostatic control of cell proliferation, cell differentiation, and programmed cell death (Trosko et al. 1998). Epigenetic characteristics of DEHP (as well as other epigenetic toxicants such as phenobarbital) include enhance mitogenesis, no-effect/threshold levels of action, and multiple and reversible toxic end points.

Hepatotoxicity and Liver Cancer. A characteristic effect of exposure to DEHP in rodents, particularly rats and mice, is an increase in liver weight, associated with both morphological and biochemical changes. Liver enlargement is due to both hepatocyte hyperplasia and hypertrophy. Morphological examination reveals an increase in both the number and the size of peroxisomes in the liver. Peroxisomes are single membrane-limited cytoplasmic organelles found in the cells from animals, plants, fungi, and protozoa. Peroxisomes contain catalase, which destroys hydrogen peroxide, and a number of fatty-acid oxidizing enzymes, one of which, acyl CoA oxidase, generates hydrogen peroxide (Lazarow and deDuve 1976). The main biochemical alterations consist of induction of both peroxisomal and microsomal fatty acid-oxidizing enzyme activities. The activity of the peroxisomal fatty acid β-oxidation cycle is normally determined either by measuring the overall activity (e.g., as cyanide-insensitive palmitoyl CoA oxidation) or by determining the first rate-limiting enzyme of the cycle, acyl-CoA oxidase. An important observation is that while the β-oxidation cycle enzymes can be greatly induced by peroxisome proliferators, other peroxisome enzymes, such as D-amino acid oxidase and catalase, are increased to a much lesser extent. As discussed below, this induction imbalance has been postulated to play a major role in phthalate-induced liver carcinogenicity. The increase of microsomal fatty acid-oxidizing enzyme activity, usually measured as lauric acid 12-hydroxylase, is due to induction of cytochrome P-450 isozymes in the CYP4A subfamily. In general, there is good correlation between enzyme activity and

changes in peroxisome morphometry (Lake 1995), allowing palmitoyl-CoA oxidation to be used as a specific biochemical marker of peroxisome proliferation.

Induction of peroxisome proliferation following treatment with DEHP is not due to the parent compound, but to DEHP metabolites. Studies with MEHP *in vitro* have demonstrated that the proximate peroxisome proliferators are mono(2-ethyl-5-oxohexyl) phthalate (metabolite VI) and mono(2-ethyl-5-hydroxyhexyl) phthalate, (metabolite IX) and that for 2-ethylhexanol, the proximate proliferator is 2-ethylhexanoic acid (Elcombe and Mitchell 1986; Mitchell et al. 1985a). Similar findings were observed by Maloney and Waxman (1999), who showed that MEHP (but not DEHP) activated mouse and human PPARα and PPARγ, while 2-ethylhexanoic acid activated mouse and human PPARα only, and at much higher concentrations. Based on its potency to induce enzyme activities, such as the peroxisomal fatty acid β-oxidation cycle and carnitine acetyltransferase, DEHP might be considered a relatively weak proliferator.

The increase in peroxisomal β-oxidation enzymes and microsomal CYP4A-associated enzymes seen shortly after administration of peroxisome proliferators is paralleled by an increase in their respective mRNAs, which is due to an increase in the transcription of their respective genes (Hardwick et al. 1987; Reddy et al. 1986). This suggested a common mechanism of induction, and raised the possibility that peroxisome proliferators act like steroid hormones by activating transcription factors (nuclear receptors) that regulate peroxisome proliferator inducible genes. Screening for nuclear receptor cDNAs in mouse liver, Issemann and Green (1990, 1991) identified a clone encoding a previously unidentified molecule, which could be activated by a variety of peroxisome proliferators, and termed it PPARα, for Peroxisome Proliferator Activated Receptor. Testing PPARα for its ability to activate gene transcription using various peroxisome proliferators revealed that there was a good correlation between the proliferators' ability to activate the receptor and their potency either as peroxisome proliferators or as rat liver carcinogens (Issemann and Green 1990; Issemann et al. 1993). Thus far, four different PPAR subtypes (α, β, γ, and δ) have been identified in various species, although only α, γ, and δ have been isolated from both rodents and humans (Cattley et al. 1998). Peroxisome proliferators regulate gene transcription through a heterodimer receptor complex composed of the PPARα and the retinoid receptor (RXR). These receptors can be activated by both peroxisome proliferators and certain fatty acids. Recently, Fan et al. (1998) showed that in acyl-CoA oxidase-deficient mice, unmetabolized long-chain acyl-CoA can function as a biological ligand of PPARα. The activated receptor complex regulates transcription by binding to DNA regulatory sequences, PP response elements (PPRE). The consensus PPRE is a direct repeat of the sequence TGACCT, located in the promoters of peroxisome proliferator responsive genes, including

those involved in peroxisomal β-oxidation of fatty acids and cytochrome P-4504A (Cattley et al. 1998; Tugwood et al. 1996). *In situ* hybridization studies in the rat have shown that PPARδ subtypes are ubiquitously expressed, whereas PPARγ is largely restricted to adipose tissue. The highest levels of expression of PPARα are observed in the liver, but there is also expression in brown adipose tissue, kidney, heart, and weakly in skeletal muscle, small intestine, testis, and thymus (Issemann and Green 1990). Human PPARα cDNA has been isolated, and encodes a functional PPARα when tested in heterologous expression studies (Mukherjee et al. 1994; Sher et al. 1993). Further information regarding the human PPAR is presented in Section 3.5.3 Animal-to-Human Extrapolations.

The role of PPAR in peroxisome proliferators-induced toxicity has been examined in several studies. For example, Lee et al. (1995) observed that PPARα-deficient mice (knockout mice) orally administered the peroxisome proliferators clofibrate or Wy-14,643 for 2 weeks did not exhibit hepatomegaly or peroxisome proliferation, and no transcriptional activation of target genes was detected. More recently, Peters et al. (1997a) fed Wy-14,643 to knockout mice lacking a functional PPARα and to normal mice. After 11 months of treatment, normal mice showed a 100% incidence of livers with multiple tumors. In contrast, in PPARα knockout mice, all livers were completely devoid of tumors. Furthermore, the same group of investigators showed that the DEHP-induced fetotoxicity and teratogenicity are not mediated through PPARα-dependent mechanisms (Peters et al. 1997b). In a study by Ward et al. (1998), treatment of PPARα wild-type mice with DEHP for up to 24 weeks resulted in typical upregulation of mRNA for peroxisomal and CYP4A enzymes in the liver and kidney, while treated null mice were no different from control wild-type or null mice. Whereas treated wild-mice had liver, kidney, and testicular toxicity, treated PPARα-deficient mice did not exhibit liver toxicity, but showed delayed moderate kidney and testicular toxicity. This suggested that while DEHP-induced liver toxicity is mediated solely by PPARα activation, both renal and testicular toxicities have both a receptor- and nonreceptor-mediated response. A study using human hepatoma cells expressing PPARα, β/δ, or γ showed that the DEHP metabolite, MEHP, activated all three isoforms of PPAR in a dose-related fashion, but DEHP did not (Lapinskas and Corton 1997). In addition, the metabolite 2-ethylhexanoic acid was isoform-specific since it activated PPARα but not β/δ or γ. Similar findings were observed by Maloney and Waxman (1999) who showed that MEHP (but not DEHP) activated mouse and human PPARα and PPARδ, while EHA activated mouse and human PPARα only, and at much higher concentrations. These data are consistent with observations *in vivo* and *in vitro* indicating that the toxicity of DEHP is due mainly to MEHP.

The exact mechanism(s) by which peroxisomal proliferating agents such as DEHP induce hepatic cancer in rodents are not precisely known, but might be related to the modulation of peroxisomal β oxidation, the

PPAR α receptor, gap junctional intercellular communication, and replicative DNA synthesis (Isenberg et al. 2000, 2001; Smith et al. 2000). Two major mechanisms have been proposed to account for peroxisome proliferator-induced hepatocarcinogenicity in rodents: induction of sustained oxidative stress and enhanced cell proliferation and promotion. Suppression of hepatocellular apoptosis has also been suggested to play a role.

Oxidative Stress. Several investigators have hypothesized that liver tumor formation arises from an imbalance between hydrogen peroxide generation and degradation within the peroxisome (Rao and Reddy 1987; Reddy and Lalwani 1983; Reddy and Rao 1989). This imbalance is the result of a much greater induction by peroxisome proliferators of hydrogen peroxide-generating enzymes than induction of catalase. This might be compounded by a reduction in enzyme activities that detoxify active forms of oxygen and organic hydroperoxides. Hydrogen peroxide that escapes the peroxisome might damage intracellular membranes and/or DNA (Reddy and Rao 1989). Lipid peroxidation and lipofuscin deposition have been observed in hepatocytes from rats treated with DEHP and other peroxisome proliferators (Cattley et al. 1987; Conway et al. 1989; Lake et al. 1987). Tagaki and coworkers have examined the possibility of DNA damage by DEHP by measuring the induction of 8-hydroxy-deoxyguanosine (8-OH-dG), a marker of DNA oxidation, in the liver and kidney from male rats administered DEHP for various periods of time (see Sai-Kato et al. [1995] for review). Increased levels of 8-OH-dG were seen in the liver after 1 or 2 weeks or 12 months of treatment, but no increases were seen in the kidney. In general, the increases were small (2-fold) and in some cases, were not sustained with prolonged DEHP treatment (Cattley and Glover 1993). Moreover, the increased levels of 8-OH-dG do not correlate with carcinogenic potency, as similar levels of induction have been associated with divergent carcinogenic activities (Marsman et al. 1988, 1992). Furthermore, DEHP and other peroxisome proliferators have consistently lacked initiation activity unlike other DNA-damaging agents. The overall evidence suggests that increased production of hydrogen peroxide and DNA oxidation are not solely responsible for peroxisome proliferator-induced liver tumor formation.

Cell Proliferation. Increased liver weight is a typical response in rodents exposed to DEHP and other peroxisome proliferators. This response is largely due to a transient increase in replicative DNA synthesis and cell division. Although considered a weak inducer of cell proliferation, DEHP causes an almost immediate increase in cell division in rats and mice (Busser and Lutz 1987; Smith-Oliver and Butterworth 1987). A single dose of 664 mg/kg DEHP produced a significant increase in DNA synthesis in the rat liver, as indicated by the incorporation of radioactive thymidine into polynucleotides during the first 24 hours (Busser and Lutz 1987). In mice, a dose of 500 mg/kg stimulated mitosis within 24 hours of

administration (Smith-Oliver and Butterworth 1987). Repeated oral doses of as little as 50 mg/kg/day increased mitotic activity when administered to rats for 3 consecutive days (Mitchell et al. 1985b). Similar findings were reported in rats and mice in a more recent study (James et al. 1998). The increase in mitosis only occurred in the early stages of treatment and did not persist beyond the first week in several exposure studies with 3–12 month durations (Marsman et al. 1988; Mitchell et al. 1985b; Smith-Oliver and Butterworth 1987). During periods of mitotic activity, there was no evidence for DNA repair according to standard repair assays (Smith-Oliver and Butterworth 1987). Increased cell division is an important factor in the tumorigenicity of both genotoxic and nongenotoxic substances because it can increase the frequency of spontaneous mutations and the probability of converting DNA adducts from both endogenous and exogenous sources into mutations before DNA can be repaired. As shown by Marsman et al. (1988), the carcinogenicity of DEHP and Wy-14,643 correlates better with sustained DNA replication than peroxisome proliferation suggesting that peroxisome proliferators induce tumors by influencing the growth of initiated lesions and therefore, act as tumor promoters. Moreover, the cell proliferation caused by peroxisome proliferators is mediated by PPARα, since treatment of wild type mice with Wy-14,643 increased S-phase, while no effect was observed in receptor knockout mice (Peters et al. 1997a).

DEHP has been examined for initiation/promotion activities in several studies. DEHP was not an initiator in the liver from mice given a single gavage dose of DEHP at 4 weeks of age, followed by treatment with phenobarbital (PB) continuously for 6 weeks; the mice were killed at 6 and 18 months (Ward et al. 1986). Similar results were reported by Williams et al. (1987), who found no evidence of induction of hepatocellular altered foci or hepatic neoplasms after treatment of rats with DEHP for 7 weeks followed by PB. Garvey et al. (1987) also found no initiating activity in the liver after single or subchronic dosing of rats with DEHP followed by PB. In promotion studies, the results have been mixed in rats, but generally positive in mice. Positive promotion activity has been reported in rat liver (Gerbracht et al. 1990; Oesterle and Deml 1988; Sano et al. 1999), rat kidney (Kurokawa et al. 1988), and mouse liver (Ward et al. 1984, 1986, 1990; Weghorst et al. 1994). Negative promotion activity was reported in rat liver (Popp et al. 1985; Ward et al. 1986; Williams et al. 1987), rat kidney (Hagiwara et al. 1990), mouse skin (Ward et al. 1986), and hamster liver and pancreas (Maruyama et al. 1994; Schmezer et al. 1988).

There is some evidence that nongenotoxic carcinogens alter apoptosis, a process that maintains the correct cell number and removes damaged cells, and that this plays a role in their carcinogenicity. As mentioned above, damaged or initiated cells (induced or spontaneous) might represent preferential targets for promotion. James et al. (1998) showed that in rat and mouse hepatocytes *in vitro*, MEHP induced DNA

synthesis and suppressed both spontaneous and TGFβ1-induced apoptosis. When tested *in vivo* in both species, DEHP significantly increased the expression of P-4504A1 and induced liver DNA synthesis and suppressed apoptosis. James et al. (1998) suggested that the carcinogenicity of nongenotoxic liver carcinogens is strongly associated with the ability to perturb hepatocyte growth regulation. Because similar results were obtained with 1,4-diclorobenzene, a chemical that is not a hepatocarcinogen in rats, James et al. (1998) suggested that the growth perturbation might need to exceed a threshold for carcinogenesis. Other *in vitro* studies with rat hepatocytes support the hypothesis that, although disruption of the mitogenic/apoptotic balance contributes to the development of DEHP-induced hepatocarcinogeneisis in rodents, a direct link between the level of oxidative stress via peroxisome proliferation and the heptocarcinogenic potential of DEHP might exist (Goll et al. 1999). Additionally, an absence of effects of DEHP on both peroxisome proliferation-associated parameters and mitogenic/ apoptotic balance supports the hypothesis that human liver cells are refractory to DEHP-induced hepatocarcinogenesis.

In summary, there is strong evidence that hepatocarcinogenesis of DEHP and other peroxisome proliferators is due to their increased production of hydrogen peroxide by peroxisomes and enhanced cell proliferation; alteration of mitogenic/apoptotic balance might also contribute. These events are triggered by the activation of gene expression via a nuclear receptor, PPARα. It should be noted that if liver cancer in humans can be promoted by DEHP via a mechanism not involving peroxisome proliferation (i.e., inhibition of gap junctional intercellular communication), the fact that this was not measured in human liver and that promotion must occur on initiated liver cells for long periods of time at a concentration that exceeds a potential threshold level (a characteristic of chemical tumor promoters) might still implicate DEHP as a potential human liver tumor promoter. However, because the model chemical, phenobarbital, is also a rodent tumor promoter and has not been shown to be a human liver tumor promoter, it is reasonable to conclude that normal exposures to DEHP will not be a significant risk factor for human liver cancers.

Reproductive Effects. DEHP induces testicular toxicity characterized by structural as well as biochemical alterations in the testis. Structural alterations consist of gross disorganization of the seminiferous tubules, with detachment of the spermatogonial cells from the basal membrane and absence of spermatocytes. Alterations in structure have been shown to be more severe in young rats than in mature animals (Dostal et al. 1988; Gray and Butterworth 1980; Sjoberg et al. 1985b, 1986a, 1986b). Structural alterations have been seen after acute- (Dostal et al. 1988; Gray and Butterworth 1980; Gray and Gangolli 1986; Oishi 1994), intermediate- (Gray and Butterworth 1980; Parmar et al. 1987; Poon et

al. 1997), and chronic-duration exposures (Ganning et al. 1991; Kluwe et al. 1982a; Price et al. 1987). Biochemical effects consisting of changes in specific activities of testicular enzymes associated with post- and premeiotic spermatogenic cells, Sertoli cells, or interstitial cells have been reported (Oishi 1986, 1994; Parmar et al. 1987, 1995).

The role of zinc in DEHP-induced testicular atrophy has been examined in several studies since a reduction in testicular zinc is a primary event following administration of DEHP. A decrease in testicular zinc, but not in serum or liver zinc, was reported in rats given DEHP (Oishi 1985; Oishi and Hiraga 1980b). After a 45-day recovery period, when there was morphological evidence of seminiferous tubule regeneration, testicular zinc was still lower than in controls (Oishi 1985). Simultaneous oral administration of DEHP and oral or intraperitoneal administration of zinc did not prevent testicular atrophy in rats, and zinc supplementation did not increase the concentration of zinc in the testis despite increases in liver and serum (Oishi and Hiraga 1983). This suggested that DEHP-induced testicular effects do not result from interference with gastrointestinal absorption of zinc, but that atrophy might be related to endogenous testicular zinc, and thus, cannot be prevented by zinc supplementation (Oishi 1985). Agarwal et al. (1986) observed a reduction in the weight of the testis, seminal vesicle, prostate, and epididymes in rats treated with DEHP and maintained in a low zinc diet, but no such effects were apparent in rats maintained in a normal or high zinc diet. Since DEHP induced effects on the liver and on serum lipids independently of the zinc concentration in the diet, Agarwal et al. (1986) concluded that the enhancement of DEHP-induced testicular toxicity in rats in a low zinc diet is limited to the testis, and confirmed that testicular atrophy is not related to zinc absorption. Zinc is thought to be localized in the spermatids; therefore, loss of zinc could be just a reflection of loss of spermatids induced by DEHP. This hypothesis was tested by Dostal et al. (1988) who treated suckling rats (<25 days of age), whose testes do not contain spermatids, with DEHP. Morphological alterations were indeed seen in the testis from the rats, suggesting that loss of zinc is not involved in the testicular atrophy at the early ages. In contrast to findings in rats, no testicular atrophy was seen in mice treated with DEHP doses that significantly reduced the zinc concentration in the testis (Oishi and Hiraga 1980a). In hamsters, DEHP did not decrease testicular zinc concentration or increase urinary excretion of zinc, as seen in rats, and caused only minor testicular alterations (Gray et al. 1982). In this case, however, the apparent lack of sensitivity of the hamster seemed to be related to a reduced rate of hydrolysis of DEHP to MEHP in the intestine, since administration of MEHP (see below for discussion regarding the active metabolite) did cause focal seminiferous tubular atrophy in hamsters (Gray et al. 1982).

The effects of DEHP on hormones that influence testicular maturation and function have also been explored. As was the case with zinc, DEHP administered to mice significantly reduced the concentration of testosterone in the testis, but no testicular atrophy was observed (Oishi and Hiraga 1980a). DEHP administered intraperitoneally to mature rats decreased the concentration of testosterone in the testis (Oishi and Hiraga 1979), but oral administration to 5-week-old rats increased the concentration of testosterone in the testis and reduced that in serum (Oishi and Hiraga 1980b). Increases in testicular concentration of testosterone along with decreases in testicular content of testosterone seen after DEHP treatment suggested that testosterone-producing Leydig cells are normal, but that the total number of cells is less than in controls or that the cells are less active in testosterone production (Oishi 1985). Simultaneous administration of DEHP and testosterone or follicle stimulating hormone (FSH) did not prevent the DEHP-induced testicular atrophy in 4-week-old rats, but did prevent the depression in accessory gland weight (Gray and Butterworth 1980). Parmar et al. (1987) reported that DEHP plus testosterone protected against DEHP-induced testicular atrophy in 10-week-old rats; however, Parmar et al. (1987) also administered a dose of testosterone 5 times higher than that used by Gray and Butterworth (1980). In a later study, Oishi (1989b) reported that co-administration of DEHP and testosterone apparently aggravated the testicular damage caused by DEHP, an effect that seemed to be due to testosterone prolonging the biological life and the mean residence time of MEHP in the testis. A mechanism for such an effect was not discussed. In a similar study, luteinizing hormone-releasing hormone significantly enhanced the testicular toxicity of DEHP when given together with DEHP (Oishi 1989a).

Mehrotra et al. (1997, 1999) presented preliminary evidence for the involvement of the thyroid and the hypophysis in DEHP-induced testicular toxicity in rats. They reported that hypothyroidism or hypophysectomy prevented, to some extent, DEHP-induced alterations in several xenobiotic-metabolizing enzymes in the testis. The significance of these findings is unclear due to a lack of DEHP-induced decreased testicular weight in sham-operated controls, which complicates comparison to the hypophysectomized animals.

Results from both *in vivo* and *in vitro* studies have indicated that the Sertoli cell is the main target for DEHP-induced testicular toxicity and that MEHP is the ultimately active testicular toxicant (Chapin et al. 1988; Creasy et al. 1986; Gray and Beamand 1984; Gray and Gangolli 1986; Sjoberg et al. 1986b). However, effects on Leydig cells have also been reported (Jones et al. 1993). The Sertoli cell is a somatic cell type whose integrity and functionality is required for the growth and maintenance of the germ cells as they divide and differentiate from spermatogonia to spermatocytes and ultimately to spermatids. The

latter are released by the Sertoli cell into the lumen as sperm. Gray and Butterworth (1980) had suggested that the Sertoli cell and not the germ cell was the direct target of DEHP toxicity since the germinal cells affected were those inside the Sertoli cell barrier. Studies by Foster et al. (1982) and Creasy et al. (1983) showed that the earliest changes after treatment with phthalates were vacuolation of the Sertoli cell followed by degenerative changes in ultrastructure in Sertoli cells, spermatocytes, and spermatids. Addition of MEHP to a mixed culture of Sertoli cells and germ cells from rat testes (4 weeks old) significantly increased the rate of germ cell detachment from Sertoli cells; no effect was seen after adding DEHP or 2-ethylhexanol (Gray and Beamand 1984). There was little effect on viability of either the germ cells or Sertoli cells with a concentration that caused germ cell detachment, although there were alterations of the Sertoli cell morphology. Also, germ cell detachment was less marked in hamsters than in rat testicular cell cultures. Testing cultures from rats of different ages showed that the enhancement of germ cell detachment produced by MEHP decreased progressively with increasing age (Gray and Beamand 1984). This indicates that, at least *in vitro*, there are factors other than pharmacokinetics that might play a role in age-related differences in susceptibility. In mixed cultures of Sertoli and germ cells, addition of MEHP caused the plasma membrane of Sertoli cells to show altered configuration (Creasy et al. 1986). Membrane disruption and mitochondrial hypertrophy were frequently observed. In primary testicular cell cultures (78–84% Sertoli cells), addition of MEHP decreased cellular ATP and the production of carbon dioxide from acetate (<60 minutes) and decreased media pyruvate, increased media lactate and intracellular lipid, and decreased mitochondrial succinate dehydrogenase (Chapin et al. 1988). All of these changes suggested that the Krebs cycle is at least one of the biochemical targets for MEHP in Sertoli cells (Chapin et al. 1988). As early as 1 hour after administration of a single oral dose of MEHP to immature rats, there were changes in Sertoli cell function as reflected by a reduction in secretion of seminiferous tubule fluid and of androgen binding protein, both specific markers of Sertoli cell function (Gray and Gangolli 1986).

Several studies have examined the role of FSH in phthalates-induced Sertoli cell toxicity. This was prompted by the fact that the initial testicular lesion in adult rats treated with dipentyl phthalate is restricted to tubules in stages with the highest FSH responsiveness (Parvinen 1982). Primary testicular cell cultures pretreated with MEHP showed a concentration-related reduction in FSH-stimulated cAMP accumulation in Sertoli cells (Lloyd and Foster 1988). This suggested a MEHP-induced perturbation at the level of the FSH membrane receptor. Further studies showed that inhibition of FSH-stimulated elevation of cAMP levels in primary rat Sertoli cell cultures required a lag period of 6 hours and did not affect the dose of FSH that gave half-maximal stimulation, suggesting that MEHP does not compete with FSH for binding to its receptor (Heindel and Chapin 1989). Later findings by Grasso et al. (1993)

revealed that in cultured rat Sertoli cells (from 18–45-day-old rats) preincubated with MEHP, there was a reduced binding of ^{125}I-hFSH to purified membrane preparations; preincubation with DEHP had no effect. The decrease in binding occurred only when MEHP was incubated with intact Sertoli cells, but not when MEHP was incubated with purified Sertoli cell membranes. Using cocultures of Sertoli cells and gonocytes from 2-day-old pups, Li et al. (1998) showed that MEHP induces germ cell detachment from Sertoli cells regardless of the presence or absence of FSH and that MEHP inhibits Sertoli cell proliferation regardless of the presence of either FSH or cAMP. No effects were seen with DEHP. Based on their results, Li et al. (1998) proposed that MEHP acts in neonatal testis either at a post-cAMP site in the FSH pathway and/or via a mechanism independent of FSH. In the study, Li et al. (1998) observed germ cell detachment with MEHP concentrations much lower than those used in other studies (Chapin et al. 1988; Gray and Beamand 1984) which confirmed that, at least *in vitro*, there is an age-related differential sensitive of Sertoli cells to MEHP. Overall, there is insufficient evidence to conclude that the mechanism of Sertoli cell toxicity involves interference of FSH signaling function by MEHP (NTP 2000b).

Alterations in Sertoli cell cytoskeleton after exposure to phthalates also have been reported. Sertoli cells form a blood-testis barrier that divides the epithelium of the seminiferous tubules into two compartments by specialized occluding tight junctions. These tight junctions are associated with actin fibers contained in the ectoplasmic specialization. Ectoplasmic specializations are plasma membranes associated with a cellular structure consisting of cells incorporating actin filament bundles, endoplasmic reticulum, and microtubules. Actin filaments in Sertoli cells play a crucial role in maintaining Sertoli cell to Sertoli cell and germ cell to germ cell interactions. A single oral dose of DEHP given to 4-week-old rats induced disruption of the ectoplasmic specialization of Sertoli cell as early as 3 hours after dosing (Saitoh et al. 1997). The lesion was characterized by marked dilation of the endoplasmic reticulum facing the tight junction and by disappearance of the actin filament bundles associated with the ectoplasmic specialization. Richburg and Boekelheide (1996) treated 4-week-old rats with a single MEHP oral dose and observed a collapse in vimentin filaments 3 hours after dosing without accompanying changes in the pattern of Sertoli cell tubulin or actin. Vimentin filaments (a type of intermediate filament) surround the Sertoli cell nucleus and extend long apical filaments which radiate toward the periphery of the cell where they associate with the plasma membrane in the region of the Sertoli-germ cell attachments (Richburg and Boekelheide 1996). These changes were correlated with an initial decrease, followed by a later increase in the normal incidence of germ cell apoptosis (programmed cell death) seen in young rats. Based on these findings, the investigators (Richburg and Boekelheide 1996) hypothesized that the Sertoli cell is responsible for directing germ cell apoptosis and that the signaling mechanism between these two cells

requires that they maintain close physical contact. Thus, MEHP-induced detachment of germ cells would uncouple the signal transduction mechanism responsible for normal cell apoptosis. Subsequent studies by the same group of investigators showed that expression of the cell surface protein Fas, an apoptosis-related system that modulates germ cell death in the testis, was highly increased in 4-week-old rats after oral exposure to MEHP (Boekelheide et al. 1998; Lee et al. 1999). Target cells undergo apoptosis when Fas ligand, a cell surface molecule belonging to the tumor necrosis factor family, binds to Fas (its receptor) (Nagata and Golstein 1995).

Exposure to DEHP during gestation and lactation has altered development of the reproductive system in male rat offspring. Oral administration of $375 mg DEHP/kg/day from gestation day 3 to postnatal day 21, or 750 mg DEHP/kg/day from gestation day 14 to postnatal day 3, has induced a variety of effects in androgen-sensitive tissues of male neonates and infants, including female-like anogenital distance and permanent nipples, vaginal pouch, penile morphological abnormalities, hemorrhagic and undescended testes, testicular and epididymal atrophy or agenesis, and small to absent sex accessory glands (Gray et al. 1999, 2000; Moore et al. 2001; Parks et al. 2000). Behavioral observations indicated that many of the exposed males were sexually inactive in the presence of receptive control females (Moore et al. 2001). The morphological effects and sexual behavioral changes are consistent with an antiandrogenic action of DEHP. Other indications of antiandrogenic activity include the lack of significant effects on time to vaginal opening and first estrus in female offspring of rats that were exposed to $375 mg DEHP/kg/day from gestation day 3 to postnatal day 21 (Moore et al. 2001). Additionally, exposure to 750 mg/kg/day from gestation day 14 to postnatal day 3 caused significantly reduced testicular testosterone production and reduced testicular and whole-body testosterone levels in fetal and neonatal male rats (Parks et al. 2000). Histological examinations of the testes in these rats showed that DEHP induced increased numbers of multifocal areas of Leydig cell hyperplasia, as well as multinucleated gonocytes, at gestation day 20 and postnatal day 3. *In vitro* assays showed that neither DEHP nor its metabolite MEHP displayed affinity for the human androgen receptor at concentrations up to 10 FM (Paganetto et al. 2000; Parks et al. 2000). The available evidence indicates that DEHP is not an androgen receptor antagonist, but acts as an antiandrogen during a critical stage of reproductive tract differentiation by reducing testosterone to female levels in the fetal male rat. Parks et al. (2000) hypothesized that DEHP, or a metabolite, reduces testosterone production either by directly acting on the Leydig cells to reduce testosterone synthesis, or by interfering with Sertoli cell paracrine factors that regulate Leydig cell differentiation and function. Regardless of the mechanism, if the Leydig cells in exposed males continue to divide rather than differentiate for only a brief period of sexual differentiation, this could delay the

onset of Leydig cell testosterone production and lead to malformations of the reproductive tract, external genitalia, and other androgen-dependent tissues (e.g., nipples) (Parks et al. 2000).

The role of gene transcription, via the PPARα, in the testicular toxicity of DEHP was examined by Ward et al. (1998). Male Sv/129 mice (6 weeks old), F_4 homozygous wild type (+/+) or knockout (-/-) for PPARα were used. Knockout (-/-) mice for PPARα lack expression of PPARα protein and are refractive to peroxisomal proliferators (Lee et al. 1995). Both strains of mice were administered DEHP in the diet at an approximate dose level of 2,400 mg DEHP/kg/day for up to 24 weeks. DEHP caused high lethality in (+/+) mice but not in (-/-) mice. All (+/+) mice showed focal tubular degenerative lesions in the testis, with diminished spermatogenesis by 8–16 weeks of treatment. In contrast, (-/-) mice had primarily normal testis after 4–8 weeks of treatment except for a few tubules in the outer portion of the testis that showed abnormal spermatogenesis. However, after 24 weeks, most (-/-) mice had severe tubular lesions. The delayed testicular toxicity of DEHP in (-/-) mice resembled the early toxicity observed in (+/+) mice and suggested that this early toxicity was mediated by PPARα. These findings indicate that PPARα is not required for DEHP-induced testicular lesions. Ward et al. (1998) speculated that other receptor subtypes (PPARδ or γ) might play a role in the delayed toxicity or that the high dose of DEHP might modify the pharmacokinetics of DEHP in the (-/-) mice. Interestingly, Peters et al. (1997b) showed that the fetotoxic and teratogenic properties of DEHP are not mediated by PPARα since effects were observed in both wild-type and PPARα-null mice treated with DEHP during gestation. Maloney and Waxman (1999) reported that PPARγ was stimulated by MEHP and not DEHP, and speculated that PPARγ could be responsible for some of the testicular effects associated with DEHP exposure. Further studies are needed to support the suggestion that activation of PPARγ is a possible mechanism for testicular effects.

Other research sought to determine whether the fetotoxic/teratogenic effects of DEHP are mediated by the PPARα (Peters et al. 1997b). Pregnancy outcome was assessed in female F_4C57BL/6N x Sv/129, wild type (+/+), and PPARα-null (-/-) mice on gestation days 10 and 18 after administration of DEHP by gavage on Gd 8 and 9. PPARα-null mice lack expression of PPARα protein and are refractive to peroxisomal proliferators (Lee et al. 1995). Relative to controls, DEHP significantly decreased the percentage of live fetuses, increased the percentage of resorptions, decreased fetal weight, and increased the percentage of fetuses with external malformations in both mice strains. On gestation day 10, maternal liver CYP4A1 mRNA was significantly elevated in DEHP-treated (+/+) mice but not in (-/-) mice, consistent with their respective phenotype. Mean maternal liver metallothionein and zinc levels were significantly higher in DEHP-treated mice (both strains) compared to controls. Maternal plasma zinc was not significantly altered as a result of treatment with DEHP. Embryonic zinc was significantly reduced in

conceptus from both mice strains. These findings indicated that DEHP-induced fetotoxicity and teratogenicity, and altered zinc metabolism are not mediated through PPARα-dependent mechanisms, and that alterations in zinc metabolism might contribute to the mechanism underlying DEHP-induced fetotoxicity and teratogenicity.

It has been hypothesized that nongenotoxic chemicals such as DEHP affect reproduction and development via the modulation (inhibition) of gap junctional intercellular communication, as proposed for liver tumor promotion (Rosenkranz et al. 2000; Trosko and Chang 1988; Trosko et al. 1998)

Gavage administration of a very high dose of 2,000 mg DEHP/kg/day to adult virgin female rats for 1–10 days resulted in prolonged estrous cycle (Davis et al. 1994a). DEHP significantly suppressed preovulatory follicle granulosa cell estradiol production, subsequently resulting in lack of luteinizing hormone (LH) surge necessary for ovulation and increases in FSH; the end result was anovulation. No other dose levels were tested in this study. The mechanism of DEHP-altered granulosa cell estradiol production was examined in cultures of rat granulosa cells exposed to the active metabolite MEHP (Davis et al. 1994b). MEHP suppressed estradiol in a concentration-related manner whether granulosa cells were stimulated with FSH or 8-bromo cyclic AMP. These findings suggested that MEHP suppressed aromatase conversion of testosterone to estradiol.

3.5.3 Animal-to-Human Extrapolations

There is ample evidence suggesting that there are species differences in both the pharmacokinetics and toxicity of DEHP; strain differences have also been described. In some cases, the differences in toxicity can be explained by differences in pharmacokinetics. The issue of greatest importance to be considered is whether DEHP can induce liver cancer and reproductive toxicity in humans, as seen in rodents. As previously mentioned, the hepatocarcinogenic response to DEHP in rats and mice is associated with peroxisome proliferation and increased hepatocyte replication. Studies in animals have shown that after exposure to peroxisome proliferators, rats and mice exhibit the greatest response, hamsters exhibit an intermediate response, whereas primates, guinea pigs, and dogs are either unresponsive or refractory (Cattley and Roberts 2000; Cattley et al. 1998; Huber et al. 1996). Studies conducted in patients treated with several hypolipidemic agents have provided no evidence for peroxisome proliferation or increased hepatocyte division (Ashby et al. 1994; Bentley et al. 1993; Cattley et al. 1998). Studies of peroxisome proliferation with primary hepatocytes *in vitro* have supported the results *in vivo*, thus validating the *in vitro* model for comparative studies. For example, Elcombe and Mitchell (1986) found that *in vitro*

exposure of rat hepatocytes to MEHP resulted in marked peroxisome proliferation and peroxisomal β-oxidation, but no such responses were observed in cultured guinea pig, marmoset monkey, or human hepatocytes, appropriately controlled for viability. In order to rule out the possibility that the species differences were due to differences in the biotransformation of MEHP, Elcombe and Mitchell (1986) isolated MEHP metabolites from rat urine and tested them in cultures from rat, guinea pig, marmoset, and human liver. Metabolite VI, biochemically and morphologically identified as the proximate proliferator in the rat, had little or no effect in marmoset, guinea pig, or human hepatocytes. These findings suggested the existence of intrinsic species differences of liver cells to peroxisome proliferators.

If peroxisome proliferation and liver carcinogenicity is mediated by PPARα, the species differences could reflect either variation in PPARα itself or in the gene networks regulated by PPARα (Green 1995). As previously mentioned, human PPARα cDNA have been isolated that encode a functional PPARα when tested in heterologous expression studies (Mukherjee et al. 1994; Sher et al. 1993). Marked species differences in the expression of PPARα mRNA have been identified. Mukherjee et al. (1994) showed that the peroxisome proliferator Wy-14,643 was a much more potent activator of the rat PPARα than the human PPARα when analyzed in CV-1 cells. However, the hypolipidemic drug clofibric acid activated equally the human and rat receptor in CV-1 cells and HepG2 cells. Palmer et al. (1998) using a sensitive immuno/DNA binding assay showed that human liver contains 10-fold lower levels of PPARα mRNA than mouse liver and that a fraction of this RNA lacks exon 6 and does not encode a functional receptor. Palmer et al. (1998) suggested that the low expression in human liver might permit PPARα to mediate some therapeutic responses to fibrates but limit the pathological changes, including peroxisome proliferation, which lead to liver cancer in rats and mice. Recently Maloney and Waxman (1999) showed that in COS-1 cells transfected with human or mouse PPARα expression plasmids and a PPRE-luciferase reporter DEHP did not activate PPARα. However, MEHP activated both human and mouse PPARα and both preparations were equally sensitive. This suggested that differential sensitivity of human PPARα cannot alone account for the lack of peroxisome proliferation response seen in humans, but other factors, such as the much lower level, as found by Palmer et al. (1998), are also likely to be important. Another important factor might be species differences in responsiveness of genes to PPARα-mediated transcription. The promoter region of the human acyl CoA oxidase gene is unresponsive to PPARα due to sequence differences from the mouse gene (Varanasi et al. 1996; Woodyatt et al. 1999)

As for testicular toxicity, which does not seem to be related to peroxisomal proliferation to the extent that liver cancer is, differential sensitivity among animal species has been found. Studies *in vivo* have shown that rats and guinea pigs are highly sensitive while mice are fairly sensitive, and hamsters and monkeys

are highly resistant (Gray et al. 1982; Kurata et al. 1998). A lack of information precludes ranking humans relative to other species. Differences in pharmacokinetics might play a role in the differential sensitivity between species, but differences in tissue sensitivity might play a role as well. Mixed cultures of Sertoli cells and germ cells from rat testes were more sensitive to MEHP toxicity than cultures from hamster testes (Gray and Beamand 1984). Also, cultures from older rats were less sensitive than cultures from young animals, suggesting that intrinsic cell factors might account for different susceptibility. Studies with the knockout mice for PPARα have suggested that other receptor subtypes (PPARδ or γ) might play a role in the delayed testicular toxicity observed in these mice or that the high dose of DEHP might modify the pharmacokinetics of DEHP in the (-/-) mice (Ward et al. 1998). Maloney and Waxman (1999) recently reported that PPARγ was stimulated by MEHP and not DEHP, and speculated that PPARγ could be responsible for some of the testicular effects associated with DEHP exposure. Further studies are needed to support the suggestion that activation of PPARγ is a possible mechanism for testicular effects. Clearly, much additional information will be necessary to determine whether the DEHP-induced testicular effects in animals are likely to occur in exposed humans.

There is information suggesting that primates may be less sensitive to DEHP than rodents and that oral absorption of DEHP is less in primates than in rodents. For example, no histopathological effects on the testes or other tissues, or other signs of toxicity, were observed in marmoset monkeys exposed to doses as high as 2,500 mg/kg/day daily for 13 weeks (Kurata et al. 1998) or in cynomolgus monkeys exposed to 500 mg/kg/day daily for 2 weeks (Pugh et al. 2000). Several studies indicate that oral absorption of DEHP is reduced in monkeys compared to rats (Albro et al. 1982a, 1982b; Astill 1989; Rhodes et al. 1986). For example, 24 hours following the last of 14 consecutive gavage doses of 2,000 mg/kg/day, tissue levels of DEHP or its metabolites were between 5 and 10 times lower in marmoset monkeys than in rats (Rhodes et al. 1986).

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors* was used by the Environmental Protection Agency (EPA) in 1996 when Congress mandated EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel

called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), which in 1998 completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Some scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

In recent years, concern has been raised that many industrial chemicals, DEHP among them, are endocrine-active compounds capable of having widespread effects on humans and wildlife (Crisp et al. 1998; Daston et al. 1997; Safe et al. 1997). Particular attention has been paid to the possibility of these compounds mimicking or antagonizing the action of estrogen, and more recently, their potential antiandrogenic properties. Estrogen influences the growth, differentiation, and functioning of many target tissues, including female and male reproductive systems, such as mammary gland, uterus, vagina, ovary, testes, epididymis, and prostate. Thus far, there is no evidence that DEHP is an endocrine disruptor in humans at the levels found in the environment.

The wealth of information in animals administered DEHP for periods ranging from a few days to lifetime studies indicate that DEHP is a developmental and reproductive toxicant by mechanisms not yet completely understood. As discussed below, the mechanisms do not appear to involve binding of DEHP to the estrogen or androgen receptors. DEHP administered perinatally to females is embryotoxic and teratogenic (reduced fetal body weight, increased rates of abortion and fetal resorptions, skeletal

malformations) (Section 3.2.2.6 Developmental Effects) and in males, it causes testicular toxicity (Section 3.2.2.5 Reproductive Effects). Whether these effects are caused by DEHP-induced hormonal disruption is not clear. As previously mentioned, a study by Davis et al. (1994a) showed that administration of 2,000 mg DEHP/kg/day to adult virgin female rats for 1–10 days resulted in prolonged estrous cycle and anovulation. Further studies in cultures of rat granulosa cells exposed to the active metabolite MEHP showed that MEHP suppressed estradiol in a concentration-related manner whether granulosa cells were stimulated with FSH or 8-bromo cyclic AMP and suggested that MEHP suppressed aromatase conversion of testosterone to estradiol. In males, the role of hormones in DEHP-induced testicular effects has also been examined, but no clear picture has emerged. DEHP administration reduced the serum level of testosterone in rats (Oishi and Hiraga 1980b) and mice (Gray and Butterworth 1980), although in mice, there was no testicular atrophy. Coadministration of DEHP and testosterone appeared to diminish, but not abolish the testicular toxicity of DEHP (Gray and Butterworth 1980). Also in rats, LH releasing hormone (LRH) significantly decreased testis weight when administered simultaneously with DEHP, while LRH and DEHP alone had no effects (Oishi 1989a). A more detailed discussion on the DEHP mechanisms of testicular toxicity is presented in Section 3.5.2, Mechanisms of Toxicity.

Early studies in experimental animals, mostly studies on pesticides, administered the chemicals orally or by parenteral routes, whereas in recent years, most of the research has focused on elucidating the mechanisms of action involved using tests systems *in vitro* which, although not without limitations, are easier to manipulate and can be developed into biomarker assays for (anti)estrogenic or (anti)androgenic activity. In general, results from *in vivo* and *in vitro* studies indicate that DEHP has negligible estrogenic potency relative to the endogenous hormone, 17β-estradiol. For example, the ability of DEHP to induce uterine wet weight and vaginal cell cornification was assessed in ovariectomized Sprague-Dawley rats (Zacharewski et al. 1998). With gavage doses of up to 2,000 mg/kg/day for 4 days, DEHP showed no consistent effects on uterine weight in duplicate experiments and did not induce vaginal cell cornification. In the same study, in an *in vitro* competitive ligand-binding assay, DEHP did not compete with 17β-estradiol for binding to the rat uterine estrogen receptor. The IC_{50} (the concentration that causes 50% response inhibition) for 17β-estradiol was 1.3 nM; an IC_{50} could not be calculated for DEHP. In assays to evaluate the effect of DEHP on estrogen receptor-mediated gene expression in transfected human breast cancer MCF-7 cells and HeLa cells, the EC_{50} for 17β-estradiol for this response was about 0.2 nM for both MCF-7 and HeLa cells; DEHP had no significant activity at the concentrations tested (0.1–10 μM). Furthermore, DEHP exhibited no estrogen receptor-mediated growth in *S. cerevisiae* at a concentration of 10 μM; 1 nM 17β-estradiol was used as positive response. The findings of Zacharewski et al. (1998) are

in good agreement with those of others (Blom et al. 1998; Harris et al. 1997; Jobling et al. 1995; Paganetto et al. 2000) in similar *in vitro* assays indicating no estrogenic activity for DEHP under the conditions of the assays.

The potential antiandrogenic properties of DEHP have been investigated in several studies. Exposure to DEHP during gestation and lactation altered development of the reproductive system in male rat offspring. Oral administration of $375 mg DEHP/kg/day from gestation day 3 to postnatal day 21, or 750 mg DEHP/kg/day from gestation day 14 to postnatal day 3, has induced a variety of effects in androgen-sensitive tissues of male neonates and infants, including female-like anogenital distance and permanent nipples, vaginal pouch, penile morphological abnormalities, hemorrhagic and undescended testes, testicular and epididymal atrophy or agenesis, and small to absent sex accessory glands (Gray et al. 1999, 2000; Moore et al. 2001; Parks et al. 2000). Behavioral observations indicated that many of the exposed males were sexually inactive in the presence of receptive control females (Moore et al. 2001). The morphological effects and sexual behavioral changes are consistent with an antiandrogenic action of DEHP. Other indications of antiandrogenic activity include lack of significant effects on time to vaginal opening and first estrus in female offspring of rats that were exposed to $375 mg DEHP/kg/day from gestation day 3 to postnatal day 21 (Moore et al. 2001). Additionally, exposure to 750 mg/kg/day from gestation day 14 to postnatal day 3 caused significantly reduced testicular testosterone production and reduced testicular and whole-body testosterone levels in fetal and neonatal male rats (Parks et al. 2000). *In vitro* assays showed that neither DEHP nor its metabolite MEHP displayed affinity for the human androgen receptor at concentrations up to 10 FM (Paganetto et al. 2000; Parks et al. 2000). The findings suggest that long-term alterations in the male reproductive system might be a consequence of perinatal exposure to DEHP. Available evidence also indicates that DEHP is not an androgen receptor antagonist, but acts as an antiandrogen during a critical stage of reproductive tract differentiation by reducing testosterone to female levels in the fetal male rat.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. 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. Relevant animal and *in vitro* models are also discussed.

Children differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their

alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Children are mainly exposed to DEHP orally from mouthing toys and other soft PVC products and possibly food, and dermally from handling materials containing DEHP. As discussed in Chapter 6 (Section 6.6, Exposures of Children), the most likely source of DEHP exposure for young children by the oral route might be toys. Other potential sources of oral exposure for young children as well as dermal exposure to all children include general household items made from PVC (e.g., dolls, plastic baby pants, furniture upholstery, floor tiles, shower curtains, tablecloths, rainwear, and shoes), which are available for mouthing by children. Oral exposure might also occur when children handle the PVC items containing DEHP and mouth their hands. Indoor exposure is possible from inhalation of both vapor and particle bound DEHP as well ingestion following inhalation of large particles containing DEHP and deposition in the upper airways and swallowing. Children's exposures to DEHP from inhalation of outdoor air is likely to be small because of the relatively low ambient concentrations. Considerable exposure to DEHP can occur from a multitude of plastic medical devices constructed from PVC, but the number of children exposed from such devices is very small compared to the population at large.

As discussed below, there is no evidence that would support the contention that children are more susceptible or predisposed to toxicity from DEHP exposure based on inherent biological differences from adults. Although they might be more highly exposed to DEHP in certain situations (e.g., during clinical procedures), for the vast general public, children are not expected to be more susceptible than adults.

No studies were located that specifically addressed effects of exposure to DEHP in children. Thus far, there is no convincing evidence of adverse health effects in humans exposed to DEHP with the exception of an early report by Shaffer et al. (1945) of mild gastric disturbances and moderate diarrhea in an adult who swallowed 10 g of DEHP. It is reasonable to expect a similar response in children exposed in a similar manner.

In animals, age might influence the susceptibility to the acute lethal effect of high doses of DEHP. Two oral doses of 2,000 mg/kg DEHP caused a high incidence of mortality in #21-day-old rats, but no mortality occurred in $42-day-old rats (Dostal et al. 1987a).

In animals, particularly rats and mice, exposure to DEHP results in liver and testicular toxicity; long-term exposures induced liver cancer (David et al. 1999; Kluwe et al. 1982a). DEHP is also embryotoxic and

has teratogenic properties (Arcadi et al. 1998; Dostal et al. 1987b; Hellwig et al. 1997; Ritter et al. 1987; Tyl et al. 1988). These effects are dose-related and have only been observed at very high doses in comparison to known or expected human exposures. Among the effects observed after perinatal administration are reduced fetal body weight, increased rates of abortion and fetal resorptions, and skeletal malformations. With regard to testicular toxicity, both *in vivo* and *in vitro* studies indicate that the Sertoli cell is the target for DEHP toxicity (Dostal et al. 1988; Grasso et al. 1993; Gray and Beamand 1984; Gray and Gangolli 1986; Poon et al. 1997; Sjoberg et al. 1986a, 1986b) and the metabolite MEHP is thought to be the active testicular toxicant. Sertoli cell alterations include vacuolization, inhibition of seminiferous tubule fluid formation, and altered testicular enzyme activities. In addition, experiments *in vitro* have shown disruption of the Sertoli-germ cell physical interaction (Li et al. 1998). There is some evidence that younger animals are more susceptible than older ones to the lethal effects of high DEHP doses (Parmar et al. 1994) and to the adverse testicular effects (Gray and Butterworth 1980; Gray and Gangolli 1986; Dostal et al. 1987a, 1988; Sjoberg et al. 1986a, 1986b). For example, testicular toxicity has been produced following oral exposure of prepubertal rats to DEHP at doses lower than those required to cause testicular effects in sexually mature rats. Some of these differences appear to be due to differences in pharmacokinetics of absorption and distribution, but there is also evidence for intrinsic differences in tissue sensitivity (Gray and Beamand 1984; Li et al. 1998). An increased permeability of the blood-testis barrier in children as compared to adults could result in increased testicular exposure to DEHP or MEHP (FDA 2001h). The blood-testis barrier forms just before puberty in humans (Furaya 1978).

DEHP altered development of the reproductive system in male rat offspring that were exposed to $375 mg/kg/day during gestation and lactation. A variety of effects were observed in androgen-sensitive tissues of young male rats, including reduced (female-like) anogenital distance and permanent nipples, vaginal pouch, penile morphological abnormalities, hemorrhagic and undescended testes, testicular and epididymal atrophy or agenesis, and small to absent sex accessory glands (Arcadi et al. 1998; Gray et al. 1999, 2000; Moore et al. 2001; Parks et al. 2000). These morphological effects, as well as reduced fetal and neonatal testosterone levels and adult sexual behavioral changes in male rats following gestational and lactational exposure, are consistent with an antiandrogenic action of DEHP. The changes in the development, structure, and function of the male reproductive tract indicate that effects of DEHP on reproduction and development are interrelated, and that long-term alterations in the male reproductive system might be a consequence of perinatal exposure to DEHP. Other evidence indicates that DEHP is not an androgen receptor antagonist, but acts as an antiandrogen during a critical stage of reproductive

tract differentiation by reducing testosterone to female levels in the fetal male rat (Paganetto et al. 2000; Parks et al. 2000).

A neurobehavioral alteration was observed in 30-day-old offspring of rats that were exposed to DEHP in the drinking water at a reported estimated dose of 33 mg/kg/day throughout pregnancy and lactation (Arcadi et al. 1998). The pups therefore were exposed both *in utero* and via breast milk. Exposure caused impaired performance in a test designed to assess locomotor activity by employing a learned avoidance task (i.e., ability to walk on a beam in order to avoid a negative stimuli). The relevance of this finding is unclear because concerns regarding the conduct of the study have been documented (particularly with respect to reliability of the dose levels) (NTP 2000b), other neurotoxicity tests were not performed (a battery of tests is needed for adequate assessment), and no effects were found in another study of DEHP that evaluated several neurodevelopmental measures. In particular, offspring of rats that were exposed to DEHP by inhalation in concentrations as high as 300 mg/m^3 for 6 hours/day on days 6–15 of gestation showed no postnatal alterations in tests of righting ability on day 6, gripping reflex on day 13, pupillar reflex on day 20, and hearing on day 21 (Merkle et al. 1988).

There is no information regarding possible transgenerational effects of DEHP in humans. A dominant lethal test in mice reported no significant effects (pregnancy rate, live fetuses, early and late fetal deaths) after treating male mice with DEHP (up to 9,860 mg/kg/day), MEHP (up to 200 mg/kg/day), or 2-ethylhexanol (up to 1,000 mg/kg/day) and then mating them with virgin females (Rushbrook et al. 1982).

No specific information was located regarding the pharmacokinetics of DEHP in children. Analysis of urine samples from humans exposed to DEHP suggests the involvement of both phase I and phase II metabolic enzymes in the biotransformation and elimination of DEHP. The specific P-450 isozymes involved in phase I metabolism are not known with certainty so no conclusions can be drawn based on general differences in isozyme activities between adults and children. Phase II reactions involve conjugation with glucuronic acid, but the specific isoform of glucuronosyltransferase is not known. Compared to adults, children generally have a reduced capacity to metabolize compounds via glucuronidation (FDA 2001h). 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. FDA (2001h) has speculated that reduced glucuronidation capacity could contribute to hepatic effects (e.g., cholestasis) observed in children undergoing ECMO therapy who were likely exposed to DEHP leached from plastic tubing used in heart-

lung bypass circuits. ECMO refers to the use of cardiopulmonary bypass to supplement blood oxygenation. The MEHP metabolite of DEHP also undergoes glucuronidation and has been shown to interfere with bilirubin conjugation (Sjoberg et al. 1991), possibly as a competitive inhibitor of glucuronidation (FDA 2001h).

DEHP has been detected in human breast milk (FDA 2001h) and therefore can be lactationally transferred from nursing mothers to children. Data are unavailable for DEHP in milk from mothers who have undergone or are undergoing medical procedures such as hemodialysis, which could result in higher levels than healthy mothers (FDA 2001h). It is well established in animals that DEHP (or metabolites) can cross the placenta and be transferred via breast milk to the offspring (Arcadi et al. 1998; Dostal et al. 1988; Hellwig et al. 1997; Peters et al. 1997b; Ritter et al. 1987; Tyl et al. 1988).

The metabolism of DEHP to the presumed toxic metabolite, MEHP, is achieved 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 2001h; Lee et al. 1993). Consequently, young children might be able to convert DEHP to MEHP more efficiently than older children or adults (FDA 2001h).

No specific information was located regarding nutritional factors that might influence the absorption or toxicity of DEHP. Because DEHP might exert toxic effects on the testes through depletion of zinc or vitamin E, and both zinc and vitamin E deficiencies are not uncommon in preterm infants due to side effects of parenteral nutrition, depletion of these substances could increase the potential for DEHP-induced testicular toxicity (Chan et al. 1999; Obladen et al. 1998; Roth et al. 1988). DEHP could also exacerbate zinc and vitamin E deficiencies that occur in preterm infants from other causes (FDA 2001h).

Unusual lung disorders were observed during the fourth week of life in children that were mechanically ventilated as preterm infants (Roth et al. 1988). The effects clinically and radiologically resembled hyaline membrane disease, a disorder caused by insufficient surfactant production in the lungs of newborn infants. Although interpretation of these findings is complicated by the preexisting compromised health status of the preterm infants, the information indicates that the lung disorders were related to DEHP released from the walls of PVC respiratory tubes. Due to its lipophilicity, it was speculated that DEHP could either inhibit the formation or promote the degradation of lung surfactant in immature lungs (Roth et al. 1988), and that this effect is less likely to be seen in adult lungs because of the increased ability of adults to produce surfactant (FDA 2001h).

There are no biomarkers of exposure or effects for DEHP that have been validated in children or in adults exposed as children. No relevant studies were located regarding interactions of DEHP with other chemicals in children or adults. No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to DEHP, reducing body burden, or interfering with the mechanism of action for toxic effects. As for adults, there are no data on methods for reducing toxic effects that might be either indicated or contraindicated in children.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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 or substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to DEHP are discussed in Section 3.8.1.

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., inhibited gap junctional intercellular communication, 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 effects caused by DEHP are discussed in Section 3.8.2.

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 3.10 "Populations That Are Unusually Susceptible".

3.8.1 Biomarkers Used to Identify or Quantify Exposure to DEHP

As discussed in Section 3.4.1, DEHP and its hydrolyzed derivatives, MEHP and 2-ethylhexanol, are absorbed from the intestinal tract, skin, and lungs into the blood (Albro 1986). Once absorbed, they are widely distributed in the body, with the liver being the major repository organ. The half-life in humans has been estimated as 12 hours (Schmid and Schlatter 1985). DEHP, MEHP, and 2-ethylhexanol are rapidly metabolized to a variety of oxidized derivatives, which are excreted in the urine and bile; some phthalic acid is also produced.

DEHP and its metabolites can be measured in the blood and urine to confirm recent exposures. Since urine samples will provide equivalent data to blood samples and can be collected using noninvasive techniques, urine samples are preferred for monitoring purposes. Analysis of the urine for DEHP is not suggested since little DEHP is excreted and measurement of DEHP in biological samples is highly subject to false positives from laboratory and sample contamination. Monitoring for MEHP, 2-ethyl-5-carboxypentyl phthalic acid, 2-ethyl-5-oxyhexyl phthalic acid, or 2-ethyl-5-hydroxyhexyl phthalic acid is more valuable since these are major urinary metabolites in humans (Schmid and Schlatter 1985). Particularly useful is a sensitive and highly selective analytical technique that enables exposure to DEHP to be specifically monitored by measuring urinary levels of MEHP (Blount et al. 2000b), and thereby avoids the ubiquitous contamination problem that limits the biomarker usefulness of direct measurements of DEHP. This analytical approach also allows specific biomonitoring of other phthalates by direct measurement of their monoester metabolites. Urinary measurements of phthalic acid can be performed, either directly or after hydrolysis to convert all phthalate derivatives to phthalic acid, but this is a nonspecific biomarker of exposure since other phthalate ester plasticizers, such as butyl benzyl phthalate, dibutyl phthalate, and diethyl phthalate, will also produce free phthalic acid after hydrolysis. Additional information on analytical methods can be found in Chapter 7.

The feasibility of using the monoester metabolites as specific biomarkers of exposure to DEHP and six other commonly used phthalates was shown in a study of urine samples collected from 289 adults during 1988–1994 as part of the Third National Health and Nutrition Examination Survey (NHANES III) (Blount et al. 2000a). The monoesters with the highest urinary levels were MEHP (95th percentile, 3,750 ppb), monobutyl phthalate (294 ppb), and monobenzyl phthalate (137 ppb), reflecting exposure to DEHP, dibutyl phthalate, and benzyl butyl phthalate, respectively. Although these measurements established a good basis for exposure biomonitoring, further calculations are needed to relate them to dose. Estimates based on the urinary MEHP measurements obtained by Blount et al. (2000a) suggest that the average total daily ambient exposure of individuals in the United States to DEHP is likely to be <3.6 µg/kg/day (David 2000; Kohn et al. 2000). Study populations larger than the 289 individuals studied by Blount et al. (2000a) are needed to gain a representative sampling of the exposure of the U.S. population to DEHP, including possible demographic variations in exposure and/or metabolism.

DEHP exposure of humans might result from intravenous administration of blood that has been stored in plastic containers, or through hemodialysis. Under situations such as these, in which DEHP is introduced directly into the blood, it is possible to evaluate exposure by measuring blood DEHP concentrations. DEHP metabolites, MEHP and phthalic acid, are also measured in the blood to determine exposure from medical products or devices (Barry et al. 1989; Sjoberg and Bondesson 1985). If the total amount of phthalate is to be monitored, the phthalate esters are first de-esterified (Liss et al. 1985). Techniques that measure total phthalic acid are not specific for DEHP exposure since other alkyl phthalic acid esters that are used as plasticizers will also produce phthalic acid after de-esterification.

DEHP is lipophilic and tends to migrate into adipose deposits. Since it is cleared from these deposits slowly, analysis of fat tissues probably provides the best test for previous exposure to this plasticizer. Analysis of human abdominal adipose tissues from accident victims indicated that DEHP was present in these tissues at a concentration of 0.3–1.0 ppm (Mes et al. 1974). DEHP was also identified in 48% of the adipose tissue specimens from cadavers autopsied in 1982 as part of the Human Adipose Tissue Survey from the National Human Monitoring Program (EPA 1989b). Neither study contained data on DEHP exposure history of the subjects, however, and there is no information regarding correlation of adipose tissue concentrations with DEHP exposure concentration and duration.

3.8.2 Biomarkers Used to Characterize Effects Caused by DEHP

Based on the animal data, the most consistent effect of exposure to DEHP is the increase in the concentration of liver peroxisomes. This effect occurs to varying degrees in all species that have been evaluated. However, evidence for an effect of DEHP exposure on human liver peroxisomes is weak. Limited data regarding biopsies from human livers, under circumstances in which DEHP was present in the hemodialysis equipment, did not lead to meaningful conclusions (Ganning et al. 1984, 1987). Therefore, a liver biopsy with subsequent histopathological examination of the cells would seldom if ever be justified as a test for the long-term effects of DEHP exposure due to the difficulties associated with this procedure.

Lipofuscin deposits in the liver might also be used as an indication of prolonged DEHP exposure based on the results of animal studies (Mitchell et al. 1985b). However, there are no data that indicate that this marker of DEHP toxicity in animal studies occurs in the human liver. In addition, lipofuscin production is not specific to DEHP; therefore a highly invasive liver biopsy to obtain a tissue sample for lipofuscin identification cannot be recommended.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Limited information was located regarding possible interactions of DEHP with other chemicals in humans. Urinary measurements of the monoester metabolites of seven common phthalates in 289 adults from the U.S. population, determined using the selective and sensitive analytical approach discussed in Section 3.8.1 (Biomarkers Used to Identify or Quantify Exposure to DEHP), showed detectable levels of monoethyl phthalate (95^{th} percentile concentration, 3,750 ppb), monobutyl phthalate (294 ppb), monobenzyl phthalate (137 ppb), 2-ethylhexyl phthalate (21.5 ppb), cyclohexyl phthalate (8.6 ppb), isononyl phthalate (7.3 ppb), and octyl phthalate (2.3 ppb), reflecting exposure to DEHP, dibutyl phthalate, benzyl butyl phthalate, di-(2-ethylhexyl) phthalate, dicyclohexyl phthalate, di-isononyl phthalate, and dioctyl phthalate, respectively (Blount et al. 2000a). Considering evidence such as this which indicates that co-exposure to multiple phthalates can occur, as well as the likelihood that many of these compounds exert effects via a common mechanism of action, there is a potential for interactions between DEHP and other phthalate esters.

Human mononuclear leukocytes were isolated from healthy young adults and incubated with 0.1–10,000 nM DEHP (Sager and Little 1989). There was no change in cell viability after 1 hour of

exposure to even the highest DEHP concentration, and no change in the binding of propyl-2,3-dihydroalprenol (DHA), a β-adrenergic blocker drug, to the α-1 glycoprotein β-adrenergic membrane binding site. DEHP did, however, displace DHA from its low affinity sites on the mononuclear leukocytes. This suggests that DEHP could potentially affect the pharmacology of the β-adrenergic class of pharmaceuticals, but further study is needed in animals before a conclusive statement can be made.

Additional information on interactions of DEHP with other chemicals was identified in animal studies. In most instances both the levels of DEHP and the interactant were high relative to potential environmental exposures.

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

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. 1982b). 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 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 indicate that it is the metabolites of DEHP that affect the enzymes rather than unmetabolized DEHP (Agarwal et al. 1982b). The authors suggest that 2-ethylhexanol acts as a competitive inhibitor of alcohol dehydrogenase when a single dose of DEHP is administered. When DEHP exposure has occurred for several days prior to ethanol exposure, the liver has adjusted to the metabolic demands of the 2-ethylhexanol. Thus, at the time of ethanol ingestion, most of the 2-ethylhexanol has been metabolized and the capacity of the liver to metabolize the ethanol has been expanded due to the induction of the alcohol metabolizing enzymes.

Data are available suggesting that DEHP might act as an antagonist for the hepatic damage caused by other chemicals. DEHP was combined with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (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 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. One possible mechanism is a reduction in TCDD-induced hyperlipidemia by DEHP stimulation of peroxisomal lipid metabolism. A second explanation is that DEHP altered the hepatic distribution of the TCDD.

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 increase in colloid droplets) and function (e.g., decrease levels of circulating T_4) (Hinton et al. 1986; Poon et al. 1997; Price et al. 1987, 1988a). When large oral doses of 500 and 2,500 mg/kg/day DEHP were combined with dietary exposure to a compound which 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 T_3 and T_4. 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 T_3 and T_4.

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 day 12 of gestation 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 2–30-fold increases in the dead and malformed fetuses and malformed survivors, but only minor decreases in the fetal weights.

Interactions between DEHP, trichloroethylene, and heptachlor on developmental toxicity have been investigated (Narotsky et al. 1995). The compounds were administered to rats by gavage on days 6–15 of gestation 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. End points that were analyzed

for possible interactions of the three chemicals included maternal death, maternal body weight gain on gestation days 6–8 and 6–20, full-litter resorption, prenatal loss, postnatal loss, pup body weight on postnatal days 1 and 6, and pups/litter with eye defects. Statistical analysis of the three maternal and six developmental end points yielded several significant two-way interactions. DEHP and heptachlor showed synergism for maternal death on gestation days 6–8 and antagonism for maternal weight gain on gestation days 6–8, full-litter resorption, and pup weight on postnatal days 1 and 6. DEHP and trichloroethylene were synergistic for maternal weight gain on gestation days 6–8, prenatal loss, and pup weight on postnatal day 6. No significant three-way interactions were observed.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to DEHP than will most persons exposed to the same level of DEHP in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters may result in reduced detoxification or excretion of DEHP, or compromised function of organs affected by DEHP. Populations who are at greater risk due to their unusually high exposure to DEHP are discussed in Section 6.7, Populations With Potentially High Exposures.

Although certain subpopulations (e.g., dialysis patients and ECMO infants) are likely to be more highly exposed to DEHP than the general population, there are few indications of people with intrinsic, biological polymorphisms or conditions that preferentially make them unusually susceptible to DEHP toxicity.

No data were located that suggest there are populations genetically at risk to DEHP toxicity, but the issue of PPAR polymorphism is beginning to be explored (Tugwood et al. 1996). However, there is strong evidence that humans, like other nonrodent species, are less susceptible than rodents to peroxisome proliferation after exposure to DEHP.

Data discussed in the sections on metabolism and reproductive effects suggest that the very young and the elderly might also bear an increased risk to DEHP toxicity if the response of humans is similar to that of rats and mice. The suggestion has been made that newborn infants with hyalin membrane disease due to immature lungs might be at risk from exposure to DEHP if they are exposed through respiration equipment during the postnatal period, due to interference with formation or turnover of alveolar surfactant (Roth et al. 1988). This possibility is not strongly supported because it is based on a limited

data base consisting of one study. Based on rodent data, testicular damage and brain damage are more likely to occur with exposures during the prenatal or early postnatal period (Arcadi et al. 1998; Cimini et al. 1994; Dabholkar 1988; Dostal et al. 1988; Tyl et al. 1988).

Physiological and pharmacodynamic changes that occur in critically ill or injured patients might place them at increased risk for developing adverse health effects following exposure to DEHP released from PVC plastic medical devices used in various procedures including blood transfusion, cardiopulmonary bypass, and ECMO (FDA 2001h). As discussed in Children's Susceptibility (Section 3.7), factors that increase the lipase-mediated metabolism of DEHP to MEHP, or the metabolism of MEHP via glucuronidation, will increase the potential for DEHP to induce adverse effects in exposed patients. Additional factors that can place patients at increased risk include increased reduced renal elimination capacity, uremia, protein malnutrition, reduced levels of antioxidants, and impaired cardiovascular status (FDA 2001h).

Limited evidence from animal studies suggests that aged rats excrete lower quantities and different proportions of DEHP metabolites than young rats (Albro et al. 1983). However, it is not clear whether age-related shifts in DEHP metabolism, or impaired liver function could exacerbate the harmful effects of DEHP on this organ.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to DEHP. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to DEHP. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

No texts were located that provided specific information about treatment following exposure to DEHP.

Most of the strategies discussed in this section will apply to high dose exposures and are consistent with guidelines generally recommended for reducing exposure to a variety of toxicants. The balance between the benefits and detriments of mitigation for low dose chronic exposures might differ from those for high dose exposures. Methods to reduce toxic effects should not be applied indiscriminately to individuals exposed to all doses of DEHP; good clinical judgement should be used.

3.11.1 Reducing Peak Absorption Following Exposure

After acute dermal or ocular exposure due to DEHP spills or other accidents, contaminated clothing should be removed and exposed skin thoroughly washed with soap and water (HSDB 2000). Exposed eyes should be flushed with a clean neutral solution such as water or saline.

A number of strategies have been suggested to minimize absorption from the gastrointestinal tract following acute, high dose ingestion. Introducing emesis is generally discouraged because DEHP can irritate the esophagus (HSDB 2000). Gastric lavage can remove DEHP from the stomach if the ingestion was recent (within 60 minutes) (HSDB 2000). Ingestion of activated charcoal is one method for reducing the intestinal absorption of DEHP since DEHP will adsorb to the carbon surface and be excreted with the fecal matter (HSDB 2000). Another method for reducing absorption is the use of a cathartic. In practice, activated charcoal is frequently given as a slurry in saline or sorbitol cathartics (HSDB 2000). Specific DEHP-binding or DEHP-reactive agents which might prevent absorption are currently not available.

3.11.2 Reducing Body Burden

In most species, including humans, DEHP is hydrolyzed in the gut to MEHP and 2-EH. Orally absorbed MEHP is probably distributed first to the liver where it is partially metabolized and then distributed to the rest of the body. Identification of the rate limiting step in metabolism and excretion of MEHP, and methods to accelerate it, might be helpful in designing future mitigation strategies. Most of the oxidized DEHP metabolites are conjugated with glucuronic acid in humans; excretion is in the bile and urine (Albro et al. 1982a). Fecal metabolites have been measured, but not quantitatively compared with metabolites secreted from the bile duct; thus it is unclear whether enterohepatic recirculation occurs. If significant enterohepatic recirculation were to be demonstrated, methods to interfere with re-uptake of DEHP metabolites into circulation might be effective in accelerating their excretion. Potential strategies for reducing intestinal resorption of bile excretions include repeated doses of activated charcoal (Levy 1982) and oral administration of the anion exchange resin, cholestyramine (Boylan et al. 1978). Neither of these approaches to promoting DEHP excretion has been studied in humans or animals, and they would only be useful for higher doses.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Significant advances have been made in recent years in the understanding of the mechanisms of liver carcinogenicity of DEHP in rats and mice. However, there is increasing mechanistic evidence suggesting that rats and mice are not an appropriate model for extrapolating to humans (Cattley et al. 1998; Doull et al. 1999; Huber et al. 1996). Therefore, speculation on how to prevent liver cancer in humans based on information in rodents seems without scientific basis.

Experiments in rats have shown that simultaneous treatment with DEHP and vitamins C and E prevents the testicular atrophy observed when rats are treated with DEHP alone (Ishihara et al. 2000). However, none of these theoretical interventions have been studied in DEHP-poisoned patients, and they are not currently recommended in human clinical medicine. The effect of a low protein diet on toxicity of DEHP in rats was addressed in a study by Tandon et al. (1992). Animals maintained on a low protein diet were more susceptible to DEHP-induced testicular damage than animals receiving a normal protein diet. However, the effects of a high protein diet were not examined and potential interactions of diet and DEHP toxicity have not been studied in humans.

Albro et al. (1989) suggests that testicular toxicity is due partially to depletion of zinc in the tissue, although it is unknown whether whole body stores are depleted by DEHP. If it were to be shown that body stores of zinc are generally depleted, then oral zinc supplementation could conceivably reduce the testicular toxicity. The success of this strategy would depend on whether the amount of oral zinc needed to increase testicular zinc levels is substantially less than that which causes zinc toxicity. Studies of zinc supplementation in rats (Agarwal et al. 1986; Oishi and Hiraga 1983) have produced conflicting data. Oishi and Hiraga (1983) found that concurrent treatment of rats with DEHP and zinc did not prevent testicular atrophy and did not increase the concentration of zinc in the testes, although the concentration of zinc in the liver and serum was increased by supplementation. In contrast Agarwal et al. (1986) found that pretreatment with a diet containing higher than normal concentrations of zinc prevented DEHP-induced reductions in body weight gain and testicular damage, but did not reduce liver damage due to DEHP. However, the relevance of the protective effects of zinc on testicular atrophy in rats is unclear because induction of testicular toxicity by DEHP in humans has not been demonstrated. Oishi (1994) found that simultaneous administration of DEHP (2,000 mg/kg/day) and the vitamin B12 derivative adenosyl cobalamine for 7 days protected against the DEHP-induced testicular toxicity in rats, but not against the liver toxicity. Also, a different vitamin B12 derivative, methyl cobalamine, was ineffective in protecting against either testicular or liver DEHP-induced toxicity.

It is not known with certainty whether any of DEHP phase I metabolites are less toxic than the others, so shifting the metabolism toward phthalic acid or a particular oxidized phthalate derivative might not change the toxicity.

Mitigation strategies developed for other peroxisome proliferators such as the fibric acid drugs should be investigated for their applicability to DEHP. Consideration should be given to the fact that other peroxisome proliferators, such as trichloroethylene, might be commonly found at the same NPL sites and toxic interactions could occur.

3.12 ADEQUACY OF THE DATABASE

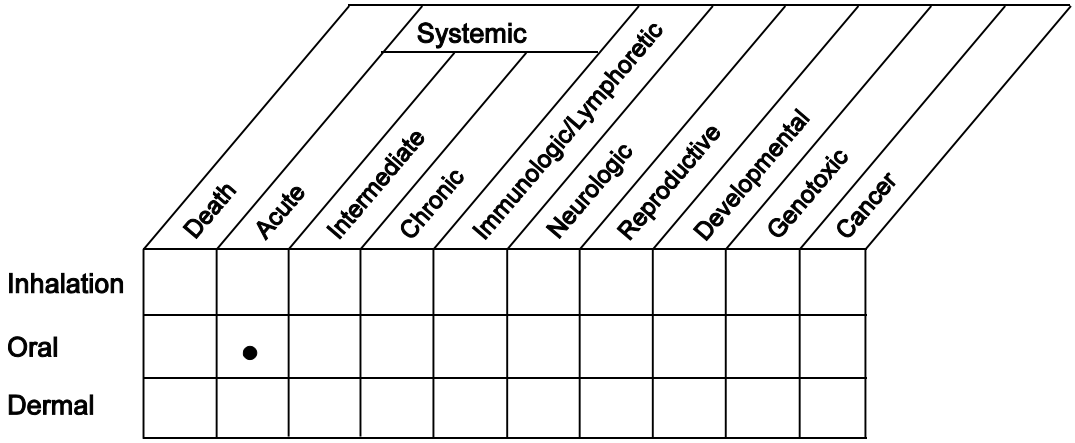
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of DEHP is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of DEHP.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

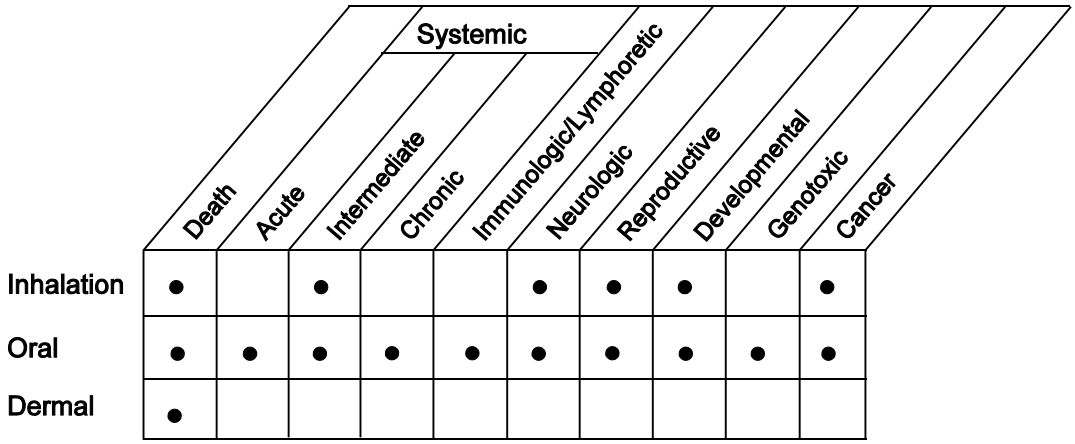
3.12.1 Existing Information on Health Effects of DEHP

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to DEHP are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of DEHP. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the

Figure 3-6. Existing Information on Health Effects of DEHP



Human



Animal

● Existing Studies

quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Few health effects have been associated with exposure to DEHP in humans as indicated in Figure 3-6. The data that exist relating to the inhalation and chronic systemic effects in humans originate from medical case studies following exposure to DEHP through respiration and hemodialysis equipment. The data are insufficient to allow for any correlation of dose and response, and there are many confounding variables that preclude reaching any conclusions concerning cause-and-effect relationships. The existing acute systemic exposure data originate from voluntary exposures of a small number of humans to daily doses of 0.01–10 g DEHP for periods of 1 or 4 days. The only parameters measured were urinary excretion and clinical signs.

The animal database on the health effects of DEHP is more complete, especially for studies using the oral route (Figure 3-6). Most studies have been conducted in rodent species, particularly rats and mice, using acute, intermediate, and chronic exposure durations. However, results are available from monkey studies as well. Systemic investigations have focused on the liver. There are limited data for the kidney, thyroid, and pancreas. There are limited data from *in vivo* studies of immune function or neurotoxicity. On the other hand, there are a number of studies that have evaluated the developmental and reproductive effects of DEHP. There is also adequate information to demonstrate that DEHP is not genotoxic in any conventional *in vivo* or *in vitro* studies of genotoxicity. The hepatic carcinogenic potential of DEHP has been clearly demonstrated in rodents.

As is apparent from Figure 3-6, there are minimal data on health effects following dermal absorption. In addition, there were only two studies on toxicokinetics that used the dermal exposure route. Although there are several animal studies that evaluated the health effects of DEHP through the respiratory route, these studies are also limited in scope. In each case, exposures were at very low levels and without effect. Although the exposure concentrations were relevant to human exposures through inhalation, the lack of observed effects makes it difficult to evaluate whether there are specific risks that apply to respiratory exposures.

As indicated above and discussed below, the health effects of DEHP are generally well characterized by the oral route in laboratory animal models. While additional information is always desirable, from a health assessment perspective, there appear to be few overriding needs for additional toxicological information for the principal route of human exposure to DEHP. Of particular importance are additional data that could enable derivation of an acute-duration oral MRL, which is currently precluded by insufficient information on male reproductive system development in offspring acutely exposed during gestation and/or lactation.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. No human data are available for acute exposures following any route except through incidental, iatrogenic exposures from medicinal practices and a limited oral study. Additional efforts to quantify these exposures and measure their effects on target tissues such as the liver and kidney would be useful when such research efforts are consistent with standard medical practices.

No data are available in animals from standard studies of acute toxicity using the inhalation or dermal routes of exposure. Therefore, an MRL value for acute inhalation exposures cannot be derived. DEHP concentrations in the atmosphere are limited by the low vapor pressure of this compound. Dermal absorption of neat DEHP is demonstrated as minimal (Deisinger et al. 1991; Melnick et al. 1987). Specific acute toxicity studies of exposures by the inhalation and dermal routes are probably not justified since exposure by these routes is thought to contribute minimally to body burden.

The liver and testes are primary targets for acute oral exposure to DEHP in adult and developing animals. When rodents are exposed to DEHP through the oral route, there is an almost immediate increase in mitotic cell division in the liver and a corresponding increase in liver weight (Barber et al. 1987; Berman et al. 1995; David et al. 1999; DeAngelo et al. 1986; Dostal et al. 1987a, 1987b; Lake et al. 1986; Lamb et al. 1987; Marsman et al. 1988; Mitchell et al. 1985b; Oishi 1989a, 1994; Parmar et al. 1988; Rao et al. 1990; Rhodes et al. 1986; Takagi et al. 1990; Tamura et al. 1990; Tomaszewski et al. 1988; Tyl et al. 1988). The number of liver peroxisomes increases and there is induction of the peroxisomal enzyme activities (David et al. 1999; Ganning et al. 1989; Rhodes et al. 1986). The activity of the MFO system is also increased (Ganning et al. 1989; Hodgson 1987; Hosokawa et al. 1994; James et al. 1998; Parmar et al. 1988; Rhodes et al. 1986; Short et al. 1987). An increase in cell division is apparent within 24 hours of administration. Further efforts to define dose-response relationships and to identify cell division and enzyme induction thresholds would be valuable. Since the pharmacokinetics and metabolism of DEHP

appear to differ between rodents and primates, it would be informative to study these phenomena in the primate. In addition to the liver, the testes (Dostal et al. 1988; Gray and Butterworth 1980; Gray and Gangolli 1986; Oishi 1986, 1994; Saitoh et al. 1997; Sjoberg et al. 1986a, 1986b) is a target tissue in rodents following DEHP exposures. Continued research to elucidate the mechanism of Sertoli cell toxicity and the role of PPARs in the reproductive toxicity of DEHP is necessary.

Acute exposure to DEHP also induces fetotoxicity and teratogenicity in rats and mice (Dostal et al. 1987b; Hellwig et al. 1997; Shiota and Mima 1985; Tomita et al. 1982a; Yagi et al. 1980). No mechanism to explain these effects has yet been proposed; thus, further studies to explore this issue are warranted. An acute oral MRL was not derived because of concern that the highest NOAEL identified (50 mg/kg/day) below all LOAELs might not be protective for testicular effects that might occur in rat pups exposed to DEHP *in utero* for acute-duration exposures. Pharmacokinetics data are available in various animal species.

Intermediate-Duration Exposure. No human data were located concerning intermediate-duration exposures of humans to DEHP by any route. No data were located from animal studies after inhalation or dermal exposures. Therefore, MRL values cannot be determined for intermediate-duration exposures by the inhalation route. Due to the low vapor pressure of DEHP and its poor dermal absorption, and the fact that the contribution of the inhalation and dermal routes of exposure to body burden is considered minimal, specific studies of intermediate-duration exposures and dose-response pattern by the inhalation and dermal routes do not appear justified.

Animal data from oral exposure studies indicate that the liver (Barber et al. 1987; David et al. 1999; Lamb et al. 1987; Mitchell et al. 1985b; Poon et al. 1997) and testes (Gray and Butterworth 1980; Lamb et al. 1987; Parmar et al. 1995; Poon et al. 1997) are main systemic targets for DEHP toxicity following intermediate-duration exposure, although some studies also reported kidney effects (Maruyama et al. 1994; Poon et al. 1997; Ward et al. 1998). Perinatal exposure to DEHP for intermediate-duration periods also caused fetotoxicity and teratogenicity in rats and mice (Arcadi et al. 1998; Cimini et al. 1994; Nikonorow et al. 1973; Shiota et al. 1980; Tyl et al. 1988). The Arcadi et al. (1998) study reported liver, kidney, and testicular alterations in neonatal rats whose mothers were exposed to DEHP during gestation and for 21 additional days postnatally. The LOAEL for this effect was much lower than those for other end points; however, this study has been judged to be inadequate for MRL derivation because the NTP-CERHR Expert Panel on DEHP (NTP 2000b) concluded that the effect levels are unreliable. In particular, NTP (2000b) found that (1) the methods used to verify and characterize the administered doses

were not clearly described or completely reported, and could not be resolved, and (2) the study authors did not reconcile their blood DEHP concentration data with other studies. Therefore, it appears that some additional dose-response studies on liver, kidney, testis, and behavioral end points would be useful, particularly to verify that the basis for the intermediate-duration oral MRL, reproductive toxicity in mice (Lamb et al. 1987), is the most sensitive end point for repeated oral exposures. Research efforts should concentrate on examining the relationship between exposure during various developmental periods and the reversibility of the effects seen in the young and trying to elucidate the mechanism(s) of fetotoxity and teratogenicity. Pharmacokinetics data are available in various animal species; however, there is lack of information regarding the pharmacokinetics of placental transfer as well as transfer of DEHP or metabolites via breast milk to offspring, although DEHP has been detected in human milk (FDA 2001h).

Chronic-Duration Exposure and Cancer. Data concerning chronic exposures of humans to DEHP were not identified in the available literature. A chronic inhalation MRL was not derived from the data from the one existing chronic animal inhalation study due to the limited number of end points that were evaluated (longevity and cancer incidence). Due to the fact that the vapor pressure of DEHP is very low and the impact on body burden of DEHP following inhalation and dermal exposure is expected to be minimal relative to oral exposure, specific studies of chronic inhalation exposures to DEHP are not recommended at this time.

There are many studies of oral chronic exposure durations in animals that indicate that the liver (Carpenter et al. 1953; David et al. 1999, 2000a, 2000b; Ganning et al. 1991; Lake et al. 1987; Marsman et al. 1988; Rao et al. 1987) and testes (David et al. 2000a, 2000b; Ganning et al. 1991; Kluwe et al. 1982a; Price et al. 1987) are main targets for DEHP. Sporadic effects in the kidneys (Crocker et al. 1988), pancreas (Rao et al. 1990), and pituitary (Kluwe et al. 1982a) also have been reported. The lowest LOAEL identified following chronic exposures is from a study where an increase in inflammation and cystic tubules were seen in the rat kidney and there was a statistically significant decrease in creatinine clearance with a gavage dose of 0.92 mg/kg/day over a 12-month period (Crocker et al. 1988). The significance of these findings is unclear due to a small number of animals, treatment of some rats with a leachate from an artificial kidney unit, limited information on kidney evaluation procedure, unreported strain and sex, and bolus method of exposure. There also was no accompanying measurement of hepatic toxicity in the exposed animals. The doses were also given for 3 of every 7 days rather than daily. No other chronic-duration study observed renal effects in rats even at much higher doses (\$600 mg/kg/day). Therefore, a more systematic evaluation of renal effects following long-term oral administration of DEHP would be useful. Perhaps of greater concern is the report of testicular toxicity in rats in a 2-year feeding

study by Ganning et al. (1991) since the testis is a known target for DEHP. Little detail was provided by Ganning et al. (1991) other than stating that the lowest dose (approximately 14 mg/kg/day) "exerted a pronounced effect on the function of the testis after prolonged treatment, consisting of inhibition of spermatogenesis and general tubular atrophy." This effect was considered a serious LOAEL, and therefore, it was not considered suitable for chronic oral MRL derivation. A chronic MRL was based on a NOAEL of 5.8 mg/kg/day for testicular pathology in male rats from a comprehensive 104-week toxicity study (David et al. 2000a).

There is no evidence that exposure to DEHP causes cancer in humans. Long-term oral administration of DEHP caused liver cancer in rats and mice (David et al. 1999; Hayashi et al. 1994; Kluwe et al. 1982a; NTP 1982). Great advances have been made in recent years with regard to the mechanism of liver carcinogenicity in rodents, particularly rats and mice, induced by peroxisome proliferators. As discussed in Section 3.5.2, Mechanisms of Toxicity, and Section 3.5.3, Animal-to-Human Extrapolations, the existing information suggests that rats and mice represent an inappropriate model for evaluating the risk of developing liver cancer by humans. The central element to developing liver cancer in rats and mice is the activation of a nuclear receptor, PPARα, which regulates the pleiotropic effects of peroxisome proliferators including the regulation of gene expression. Activation of PPARα leads to increased activity of peroxisomal enzymes of β-oxidation and of microsomal enzyme cytochrome P-4504A1. This is accompanied by increased cell replication which, along with increased production of hydrogen peroxide, are proposed to be the main mechanisms of peroxisome proliferator-induced hepatocarcinogenesis (Cattley et al. 1998; Doull et al. 1999; Green 1995; Lake 1995). Humans have low liver expression of PPARα and are refractory to peroxisome proliferators, and so are primates. Future research should focus on (1) further characterization of the human PPARα and its tissue distribution, (2) studies of mechanisms by which the low receptor expression could be altered (increased) resulting in human responses to peroxisome proliferators similar to rodents, (3) better understanding on how peroxisome proliferators and fatty acids activate PPRAα, (4) examination of possible polymorphism of PPARs in populations of different ethnic backgrounds, (5) characterization of the interaction between hypolipidemic drugs and PPARα resulting in reduced serum triglycerides, but no stimulation of peroxisome proliferation, and (6) characterization of other PPAR subtypes, such as PPAR γ, and their tissue distribution and role in other DEHP-induced toxicities, such as reproductive toxicity.

Populations occupationally exposed to DEHP as well as subjects exposed through medical devices should continue to be monitored for liver effects.

Additional bioassays in animals do not seem necessary. Further research on dose-response relationships for the many biochemical effects of peroxisome proliferators leading to liver cancer in rodents, identification of specific thresholds, and potential reversibility, would be informative only if an extrapolation model for cancer was deemed appropriate in spite of profound differences between human and rodent responses.

Genotoxicity. The genotoxicity of DEHP and its primary metabolites, MEHP and 2-ethylhexanol, has been extensively evaluated in most standard short-term tests of genetic toxicity. The data are consistent and indicate that DEHP, MEHP, and 2-ethylhexanol are not genotoxic.

The role of oxidative DNA damage, as measured by induction of 8-hydroxydeoxyguanosine (8-OH-dG), in the liver of rats following DEHP exposure has been examined by Takagi and coworkers (Sai-Kato et al. 1995). While increased induction of 8-OH-dG was demonstrated, the increased was small (2–3-fold) and, in some cases, was not sustained during prolonged treatment (Cattley and Glover 1993). Additional studies might be valuable to clarify whether nuclear DNA is, in fact, oxidized following treatment with DEHP by analyzing repair enzyme activity and/or excised base levels in the urine, along with oxidative DNA products other than 8-OH-dG. Studies to compare 8-OH-dG levels in purified nuclear DNA from DNA of mitochondria from hepatocytes and nonparenchymal liver cells from control and DEHP-treated rats would resolve the issue of whether the 8-OH-dG issue is relevant to DEHP genotoxicity or mitogenesis. These data would provide additional information on whether or not free radical oxidation resulting from peroxisome proliferation plays a role in the carcinogenesis of DEHP in rodents.

Reproductive Toxicity. There are no reported reproductive effects of DEHP in humans, but there is ample evidence that DEHP has adverse effects on reproductive effects in rats and mice. In males, exposure to DEHP affects the weight of the male reproductive organs and the process of spermatogenesis (Dostal et al. 1988; Ganning et al. 1991; Gray and Butterworth 1980; Gray and Gangolli 1986; Lamb et al. 1987; Oishi 1986; Parmar et al. 1995; Poon et al. 1997; Saitoh et al. 1997; Shaffer et al. 1945; Sjoberg et al. 1986a, 1986b; Ward et al. 1998). Effects are seen after acute-, intermediate-, and chronic-duration exposure, and NOAELs for testicular and reproductive toxicity were used as the basis of the intermediate and chronic oral MRLs as discussed in Chapter 2 and detailed in Appendix A. The effects of DEHP are most severe when they occur during the process of male sexual organ development or maturation, and thus are age related (Arcadi et al. 1998; Dostal et al. 1988; Gray and Butterworth 1980; Gray et al. 1999, 2000; Moore et al. 2001; Parks et al. 2000; Sjoberg et al. 1985b, 1986a, 1986b). However, the testicular changes appear to be reversible if DEHP exposure ceases before puberty (Dostal et al. 1988). The data on

the testicular toxicity of DEHP indicate that the Sertoli cell is the main target and that MEHP is the ultimate active testicular toxicant (Chapin et al. 1988; Creasy et al. 1986; Gray and Beamand 1984; Gray and Gangolli 1986; Sjoberg et al. 1986b). Since pharmacokinetic data indicate that DEHP is converted to MEHP primarily in the gastrointestinal tract, oral studies seem most relevant. Additional research on the mechanism of Sertoli cell toxicity and on the characterization of PPARγ and its role in DEHP-induced reproductive toxicity is necessary (Maloney and Waxman 1999; Peters et al. 1997b). Further studies that directly examine the susceptibility of PPARγ target genes in various tissues and species to MEHP would be valuable. A physiologically based pharmacokinetic (PBPK) model of DEHP in rats that simulates the pharmacokinetics of both DEHP and its major metabolite, MEHP was recently described (Keys et al. 1999). The model provides an approach to estimating doses of MEHP in the testes of the rat following oral doses of DEHP and might be useful for internal dose-response assessment of rat bioassay data in which the toxicity end point of interest is testicular toxicity. However, such uses of the model, or other potential uses in risk assessment, have not been evaluated.

DEHP altered development of the reproductive system in male rat offspring that were exposed during gestation and lactation. A variety of effects were observed in androgen-sensitive tissues of young male rats, including reduced (female-like) anogenital distance and permanent nipples, vaginal pouch, penile morphological abnormalities, hemorrhagic and undescended testes, testicular and epididymal atrophy or agenesis, and small to absent sex accessory glands (Arcadi et al. 1998; Gray et al. 1999, 2000; Moore et al. 2001; Parks et al. 2000). These morphological effects, as well as reduced fetal and neonatal testosterone levels and adult sexual behavioral changes in male rats following gestational and lactational exposure, are consistent with an antiandrogenic action of DEHP. The changes in the development, structure, and function of the male reproductive tract indicate that effects of DEHP on reproduction and development are interrelated, and that long-term alterations in the male reproductive system might be a consequence of perinatal exposure to DEHP. Other evidence indicates that DEHP is not an androgen receptor antagonist, but acts as an antiandrogen during a critical stage of reproductive tract differentiation by reducing testosterone to female levels in the fetal male rat (Paganetto et al. 2000; Parks et al. 2000). Because the dose-response relationships for reproductive effects following exposures in gestational versus postnatal ages are unknown, low-dose studies examining sensitive end points following late gestational exposure are a critical data need.

The mechanism of the effect of DEHP on the female reproductive processes has not been studied as extensively as that of the male. Female mice orally exposed to high doses of DEHP (420 mg/kg/day) failed to produce any litters when mated with control males (Lamb et al. 1987). DEHP affected female

fertility and pup survival in cases where pregnancy was achieved. A reproductive toxicity NOAEL of 14 mg/kg/day from this study was used as the basis of the intermediate oral MRL. Information on the dose-response relationship for these effects as reflected in DEHP's impact on ovulation, implantation, and the early stages of gestation would be useful. It would also be useful to know if the metabolite responsible for the female reproductive effects is 2-ethylhexanoic acid, the metabolite responsible for the developmental effects, or a different DEHP degradate. Davis et al. (1994a) showed that DEHP induced anovulation in virgin adult rats by altering granulosa cell estradiol production. Further studies with cultures of granulosa cells *in vitro* suggested that MEHP suppressed aromatase conversion of testosterone to estradiol (Davis et al. 1994b). The possible role of these findings in fertility changes in females needs to be explored. In marmoset monkeys, 2,500 mg/kg/day DEHP by oral administration for 13 weeks did not cause any gross or microscopic effects in ovary, uterus, or vagina (Kurata et al. 1998).

Developmental Toxicity. There are no data concerning developmental effects in humans following DEHP exposures. There are animal data for exposures by the inhalation (Merkle et al. 1988) and oral routes (Arcadi et al. 1998; Cimini et al. 1994; Dostal et al. 1987b; Hellwig et al. 1997; Peters et al. 1997b; Price et al. 1986, 1988c; Ritter et al. 1987; Shiota and Mima 1985; Shiota et al. 1980; Tomita et al. 1982a; Tyl et al. 1988; Yagi et al. 1980). There are no dermal data in animals. DEHP is a teratogen in rats and mice when given orally during the gestation period. Mice appear to be more vulnerable to the teratogenic effects of DEHP than rats (Tomita et al. 1982a; Tyl et al. 1988). Studies in mice using single dosing during one of several gestation days identified day 7 of gestation as the most sensitive (Tomita et al. 1982a). Malformations of the skeleton and neuronal tube were commonly observed. The DEHP metabolite responsible for the teratogenic effects appears to be 2-ethylhexanoic acid (Hauck et al. 1990; Ritter et al. 1987). Furthermore, it has been suggested that only the (R) enantiomer of this compound acts as a teratogen (Hauck et al. 1990). The mechanism of fetotoxicity/teratogenicity of DEHP (or metabolites) has not been elucidated. Results from a study by Peters et al. (1997b) in mice suggested teratogenicity and fetotoxicity of DEHP was not mediated by the nuclear receptor PPARα. Further research into the possible role of other receptor subtypes could provide valuable information on the mechanisms underlying the developmental toxicity of DEHP. Studies in primates would be particularly relevant to humans. Data needs related to effects of DEHP on the development of the male reproductive system are discussed in the previous section. Since the developmental effects of DEHP in animals might be caused by a metabolite(s) resulting from chemical reactions that follow oral exposure, inhalation or dermal studies do not seem necessary at this time.

There is lack of information regarding the pharmacokinetics of placental transfer as well as transfer of DEHP or metabolites via breast milk to the offspring, although DEHP has been detected in human milk (FDA 2001h).

Immunotoxicity. There are currently no *in vivo* studies in humans or animals that examined immunocompetence following exposure to DEHP by any route of exposure. No histopathological alterations to organs of the lymphoreticular system due to treatment with DEHP were reported by any animal study. DEHP does bind to mononuclear leukocytes *in vitro* (Sager and Little 1989) but this is not an inherently toxic phenomenon. The overall evidence suggests that the immune system is not a target for DEHP toxicity. Specific studies addressing this issue do not seem necessary at this time.

Neurotoxicity. There are no data concerning neurotoxic effects in humans following DEHP exposure by any route; data in animals are very limited. Moser et al. (1995) found no evidence of neurotoxicity in rats following administration of a single dose of up to 5,000 mg DEHP/kg of daily dose of up to 1,500 mg DEHP/kg for 14 days. Signs of general debilitation seen at 5,000 mg/kg cannot be categorized as specific signs of neurotoxicity. Tests conducted by Moser et al. (1995) assessed autonomic, sensorimotor, and neuromuscular functions, as well as excitability and activity. No neurological effects were reported in any long-term study. Data from developmental studies in animals indicate that DEHP interferes with normal development of the nervous system in rodents (Hellwig et al. 1997; Shiota and Mima 1985; Shiota et al. 1980; Tyl et al. 1988; Yagi et al. 1980). Exencephaly and neural tube defects have been seen in several studies that have evaluated the effects of DEHP administered during gestation. Also, in a study in female rats given DEHP in the drinking water from gestation day 1 to day 21 after delivery, female pups showed neurobehavioral impairment when tested at 30 days of age (Arcadi et al. 1998). This finding is unique to this study and replication of the results would greatly increase the confidence in the study. Studies should be designed to elucidate the mechanism(s) for this effect. In addition, DEHP exposure through maternal milk was associated with an increase in brain peroxisomes in newborn rats (Cimini et al. 1994; Dabholkar 1988). A significant observation of Cimini et al. (1994) was the fact that brain catalase activity doubled in adult dams treated with DEHP, but was not significantly changed in the pups. This led them to suggest that immature neural cells are not yet able to modulate this activity and are therefore more susceptible to oxidative stress than mature ones. Thus, research concerning the role of the peroxisomes in the developing brain and the effect of DEHP induced changes in the activity of the brain peroxisomes would be beneficial. Of particular interest would be knowledge as to whether or not DEHP causes a modification of the brain lipids, particularly those of the myelin sheath, through oxidative processes. Changes in membrane proteins in the brain could also be studied since receptor site

interactions are so important in brain development and function. Additional studies in adult animals do not seem necessary at this time based on the lack of effects in long-term studies. Also, structurally, DEHP does not appear to be a neurotoxicant.

Epidemiological and Human Dosimetry Studies. Information on health effects of DEHP in humans is essentially limited to observations of gastrointestinal distress in two individuals who ingested a single large dose of the compound (Shaffer et al. 1945). Repeated dose oral studies in rats and mice have established that the main targets of DEHP toxicity are the liver and testes. In contrast to the findings in rats and mice, monkeys appear to be relatively insensitive to the hepatic and testicular effects of DEHP (Kurata et al. 1998; Rhodes et al. 1986; Short et al. 1987). Sustained long-term oral exposure to DEHP is hepatocarcinogenic in rats and mice, but the mechanism by which liver cancer (and liver toxicity) is induced in these species does not appear to be operative in humans (David et al. 1999; Kluwe et al. 1982a; Rao et al. 1987, 1990). The available data indicate that DEHP is unlikely to cause adverse health effects in environmentally exposed humans and therefore do not establish a clear need for epidemiological and human dosimetry studies. Identification and follow-up studies of children who were heavily exposed to DEHP, particularly the evaluation of reproductive system development and function in premature infants with still developing testicles exposed via plastic devices during medical procedures, could address the issue of whether there are functional effects in the most heavily exposed and vulnerable human population; however, such studies are not relevant to general population exposure due to the intensive and unnatural nature of the intravenously or and/or intratracheal procedures.

Biomarkers of Exposure and Effect. There may be no particular benefit in better characterizing biomarkers for DEHP because (1) given its ubiquity (albeit at low environmental levels), there appears to be no great need to ascertain whether humans have been exposed (unless in known, high-exposure situations), and (2) there is no confirmed or compelling evidence that ambient exposure to DEHP can harm human health.

Exposure. Because DEHP is rapidly metabolized and excreted, it is difficult to monitor anything but recent human exposures through the body fluids. MEHP and several oxidized MEHP metabolites can be measured in blood and urine and are biomarkers of exposure, and DEHP has been detected in human milk. Since DEHP is a lipophilic substance, it has the potential to deposit in adipose tissues. More chronic exposures can be detected with a fat biopsy, but there are no validated approaches for assessment of chronic exposure by fat biopsy analysis. Additional studies of methods for monitoring DEHP exposure would be of value.

Effect. Currently there are no simple methods of measuring the effects of DEHP exposure. An increase in liver peroxisomes and peroxisomal enzyme activities appears to be the best marker of effect in rodents. This is not of great value in human studies since there is extensive evidence that humans, as well as primates, are refractory to peroxisome proliferators. Accordingly, research to identify reliable biomarkers for DEHP effects in humans would be useful in order to evaluate the prevalence and magnitude of exposure in an at-risk population.

Absorption, Distribution, Metabolism, and Excretion. There are no data on the absorption, distribution, and excretion of DEHP following inhalation exposure of humans. The only human data apply to the urinary excretion of DEHP metabolites following oral exposures. In animals, there are data pertaining to the dermal and oral routes. Dermal data were provided in studies by Melnick et al. (1987) and Deisinger et al. (1998) in rats and suggested that dermal absorption is limited. DEHP is hydrolyzed by esterases found in a variety of tissues, although pancreatic lipase is the most effective enzyme hydrolyzing DEHP. Therefore, DEHP is absorbed primarily as MEHP and 2-ethylhexanol (Albro 1986). With high exposure concentrations, some DEHP might also be absorbed. DEHP metabolites are distributed to tissues via the blood. MEHP and 2-ethylhexanol are metabolized to a variety of more oxidized compounds. The liver is the main target tissue for DEHP metabolites (Ikeda et al. 1980; Rhodes et al. 1986). In species other than the rat, some of the oxidized intermediates are conjugated with glucuronic acid for excretion (Albro et al. 1982a). These metabolites are excreted in the urine and bile. Unabsorbed DEHP and MEHP are excreted in the feces. Additional data on the identity of the metabolites excreted in the bile and the portion that is excreted as conjugates would be useful in interpreting the absorption and metabolic data. Studies of dermal absorption from various vehicles are also justified since this has never been examined and the vehicle might have a profound effect on absorption.

Comparative Toxicokinetics. The toxicity of DEHP differs among species. This is due both to differences in pharmacokinetics and species-specific differences in target tissue susceptibilities. For example, there are species differences in the rate of hydrolysis of DEHP to MEHP in the intestine. Hydrolytic activity is highest in the mouse, followed by the rat, guinea pig and hamster (Albro 1986; Lake et al. 1984a). Hydrolysis in primates and in humans is considerably slower than in rats (Albro et al. 1982a; Rhodes et al. 1986). This of great importance because MEHP is the active peroxisome proliferator. The proportions of the different metabolites excreted and the proportions of metabolites that are excreted as conjugates vary (Albro et al. 1982a, 1982b, 1987; Astill 1989; Rhodes et al. 1986; Short et al. 1987). Primates glucuronidate the oxidative metabolites of MEHP more completely, while oxidizing

metabolites less effectively, and rats do not glucuronidate DEHP metabolites. There are only a few studies on primate and canine species and none of these were rigorous. Primate studies are especially important in light of the fact that rodents have been, and will continue to be, the subject of much research due to their suitability as a model for the peroxisome proliferation phenomenon. Additional data on DEHP metabolites which occur in humans would also be useful in evaluating the potential for health effects to occur following DEHP exposures.

Methods for Reducing Toxic Effects. There are no established methods for reducing absorption of DEHP or metabolites because the mechanism of absorption is not known. There have been no studies of compound-specific techniques for reducing DEHP body burden. External contact with DEHP can be treated by thoroughly washing the affected area. Activated carbon, possibly combined with a cathartic, will diminish absorption of ingested DEHP from the gastrointestinal tract (HSDB 2000).

There are no tested methods for preventing or minimizing the toxic effects of DEHP. Currently, there are no records of cases of high-dosage human exposure to DEHP, aside from an early toxicology experiment (Shaffer et al. 1945). If situations leading to high-dose exposures are identified, research on minimizing acute toxic effects would be important. A study of the impact of dietary modifications (increased intake of antioxidants, zinc and glutathione precursors, and decreased dietary fat) on the effects of chronic exposure to DEHP might be useful. Given that an *in vivo* rodent study has shown that antioxidant green tea components could reduce the inhibitory effects on gap junctional intercellular communication by pentachlorophenol, a nongenotoxic liver tumor promoter, and because DEHP also has been shown to inhibit gap junctional intercellular communication (Malcolm and Mills 1983, 1989), a study designed to see if green tea or its antioxidant components could reduce the liver tumor promoting activity of DEHP would be warranted.

Children's Susceptibility. There is virtually no information on the health effects of DEHP in humans. Most studies in animals have been conducted in rodents, particularly rats and mice. In these species, DEHP causes testicular toxicity (Dostal et al. 1988; Gray and Butterworth 1980; Gray and Gangolli 1986; Poon et al. 1997; Saitoh et al. 1997; Sjoberg et al. 1986a, 1986b), is fetotoxic and teratogenic (Arcadi et al. 1998; Dostal et al. 1987b; Hellwig et al. 1997; Ritter et al. 1987; Tomita et al. 1982a; Tyl et al. 1988), and induces liver cancer (David et al. 1999; Kluwe et al. 1982a). The rats and mice in which DEHP has induced liver cancer do not appear to represent an accurate model for human carcinogenicity (IARC 2001) and there is no information on whether the developmental process is altered in humans exposed to DEHP. There is no evidence that DEHP has hormone-like effects, but limited

information from one study using one high dose (750 mg/kg/day) indicates that DEHP might have antiandrogenic properties in male pups from rats given DEHP during gestation and for few days during nursing (Gray et al. 1999). Further studies are necessary to characterize the dose-response and examine potential interactions of DEHP (or metabolites) with the androgen receptor.

There are no adequate data to evaluate whether pharmacokinetics of DEHP in children are different from adults. It is not known whether DEHP (or metabolites) can cross the placenta in humans, although it has been detected in breast milk (FDA 2001h). Studies in animals have shown that DEHP (or metabolites) crosses the placenta and can be transferred to offspring via mother's milk; however, quantitative data are lacking. There is no information to evaluate whether metabolism of DEHP is different in children than in adults since the specific phase I enzymes involved in DEHP metabolism have not been identified. It is known that phase II metabolism involves conjugation with glucuronic acid, but the specific isoform of glucuronosyltransferase is not known.

There is no information about whether children differ in their susceptibility to the health effects of DEHP. However, studies in animals indicate that the younger the animal, the more severe the testicular effects induced by DEHP (Gray and Butterworth 1980; Sjoberg et al. 1986a, 1986b) This differential susceptibility is partly related to differences in pharmacokinetics (Sjoberg et al. 1985b), but other, yet undetermined factors, also might play a role (Gray and Beamand 1984). One study in which rats were exposed during gestation and lactation reported altered neurological responses in female offspring tested at 30 days of age (Arcadi et al. 1998). These findings need to be replicated by others, and if so, research efforts should focus on the possible underlying mechanism(s) that are responsible for such alterations.

Continued research into the development of sensitive and specific early biomarkers of exposure and effect for DEHP would be valuable for both adults and children. There are no pediatric-specific methods to reduce peak absorption for DEHP following exposure, to reduce body burdens, or to interfere with the mechanism of action. Based on the information available, it is reasonable to assume that methods recommended for treating adults will also be applicable to children.

Child health data needs relating to exposure are discussed in Section 6.8.1 Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Several ongoing studies concerning health effects associated with DEHP have been identified in the Federal Research in Progress (FEDRIP 2001) and are listed in Table 3-7.

Table 3-7. Ongoing Studies on the Health Effects of DEHP

Investigator	Affiliation	Research description	Sponsor
Jirtle RL	Duke University Medical Ctr. Durham, North Carolina	Tumor suppressor function of the M6P/IGF2 receptor	NIEHS
Lied M	Oregon State University Corvallis, Oregon	Molecular determinants of peroxisomal proliferator action	NIEHS
Orth JM	Temple University School of Medicine Philadelphia, Pennsylvania	Mechanism of toxicant induced injury in neonatal testes	NIEHS
Richburg JH	University of Texas Division of Pharmacology and Toxicology, Austin, Texas	Environmental testicular toxicity and germ cell apoptosis	NIEHS
Swenberg JA, Thurman RG	University of North Carolina Chapel Hill, North Carolina	Lipid metabolism and phthalate toxicity interactions	NIEHS

Source: FEDRIP 2001

NIEHS = National Institute of Environmental Health Sciences