

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 di-*n*-butyl phthalate. 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. 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

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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 (LOAEL) 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.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for di-*n*-butyl phthalate. 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 1990b), 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.

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3.2.1 Inhalation Exposure**3.2.1.1 Death**

No studies regarding death in humans following inhalation exposure to di-*n*-butyl phthalate were located.

Studies in rats calculate acute inhalation LC₅₀ values for rats of 25 g/m³ (NTP 1995) and 4.25 g/m³ (NTP 1995), and for mice of 25 g/m³ (NTP 1995). No other studies regarding death in animals following inhalation exposure to di-*n*-butyl phthalate were located.

3.2.1.2 Systemic Effects

No studies regarding gastrointestinal, musculoskeletal, endocrine, dermal, or ocular effects in humans or animals following inhalation exposure to di-*n*-butyl phthalate were located.

Respiratory Effects. No studies regarding respiratory effects in humans following inhalation exposure to di-*n*-butyl phthalate were located.

Information on the potential of di-*n*-butyl phthalate to induce respiratory effects is limited to a study by Kawano (1980a), which found an increase in relative lung weight in rats exposed to 50 mg/m³ di-*n*-butyl phthalate 6 hours/day for 6 months (Kawano 1980a); lung weight was not altered in the 0.5 mg/m³ group. The toxicological significance of the altered lung weight is difficult to assess in the absence of a study that examined histopathology.

Walseth and Nilsen (1984) reported a decrease in lung cytochrome P450 in rats exposed to 28.4 or 79.5 mg/m³ but not 5.7 mg/m³, di-*n*-butyl phthalate for 5 of 6 consecutive days, although no statistical significance was indicated.

Cardiovascular Effects. There is a limited amount of information available on the cardiovascular toxicity of di-*n*-butyl phthalate in humans. Hypertension was reported in workers exposed to di-*n*-butyl phthalate for 0.5–19 years at concentrations of 1.7–66 mg/m³; the frequency increased with length of employment (Milkov et al. 1973). These workers were also exposed to other plasticizers, so the effects seen may not have been caused by di-*n*-butyl phthalate exposure.

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Hematological Effects. No studies regarding hematological effects in humans following inhalation exposure to di-*n*-butyl phthalate were located.

No alterations in hematocrit, hemoglobin, or erythrocyte levels were observed in rats exposed to 50 mg/m³ di-*n*-butyl phthalate 6 hours/day for 6 months (Kawano 1980a).

Hepatic Effects. Data on the hepatotoxicity of di-*n*-butyl phthalate is limited to a report of hyperbilirubinemia among workers exposed to 1.7–66 mg/m³ for 0.5–19 years; the frequency increased with length of employment (Milkov et al. 1973). These workers were also exposed to other plasticizers, so the effects seen may not have been caused by di-*n*-butyl phthalate exposure.

Relative liver weight was not affected in rats exposed to 50 mg/m³ di-*n*-butyl phthalate mist 6 hours/day for 6 months (Kawano 1980a). Small fluctuations in several serum chemistry parameters (serum enzymes, urea nitrogen, cholesterol) were noted in this study, but these were not clearly dose- or time-dependent.

Renal Effects. No studies regarding renal effects in humans following inhalation exposure to di-*n*-butyl phthalate were located.

Relative kidney weight did not differ from controls in rats exposed to 50 mg/m³ di-*n*-butyl phthalate 6 hours/day for 6 months (Kawano 1980a).

Endocrine Effects. No studies regarding endocrine effects in humans or animals following inhalation exposure to di-*n*-butyl phthalate were located.

Body Weight Effects. No studies regarding body weight effects in humans following inhalation exposure to di-*n*-butyl phthalate were located.

An approximate 13% decrease in body weight gain was observed in rats exposed to 50 mg/m³ di-*n*-butyl phthalate 6 hours/day for 6 months (Kawano 1980a).

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3.2.1.3 Immunological and Lymphoreticular Effects

No studies regarding immunological effects in humans following inhalation exposure to di-*n*-butyl phthalate were located. Small fluctuations in white cell counts and percent neutrophils were found in rats exposed to 0.5 and 50 mg/m³ di-*n*-butyl phthalate 6 hours per day for 6 months, but the changes were not dose- or time-dependent (Kawano 1980a), and do not appear to be clinically significant.

3.2.1.4 Neurological Effects

Workers exposed to di-*n*-butyl phthalate for 0.5–19 years at concentrations of 1.7–66 mg/m³ experienced neurological symptoms (pain, numbness, spasms, weakness) and exhibited reflex disturbances, elevated thresholds for pain sensitivity and olfactory stimulation, and depression of vestibular function (Milkov et al. 1973). The frequency and severity of these effects increased with increased duration of exposure. The workers were also exposed to other plasticizers, so these neurological effects may not have been caused by di-*n*-butyl phthalate exposure.

In rats, a statistically significant increase in brain weight as a percent of body weight was observed following exposure to 50 mg/m³ di-*n*-butyl phthalate for 6 months (Kawano 1980a). However, a significant decrease in body weight gain was reported for this dose group, and the absolute brain weight increase was small (1.58 g versus 1.47 g in controls).

3.2.1.5 Reproductive Effects

No studies regarding reproductive effects in humans following inhalation exposure to di-*n*-butyl phthalate were located. In rats, exposure to 0.5 or 50 mg/m³ 6 hours/day for 6 months caused no changes in relative testicular weight (Kawano 1980a).

3.2.1.6 Developmental Effects

No studies regarding developmental effects in humans or animals following inhalation exposure to di-*n*-butyl phthalate were located.

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3.2.1.7 Cancer

No studies regarding cancer effects in humans or animals following inhalation exposure to di-*n*-butyl phthalate were located.

3.2.2 Oral Exposure

Table 3-1 and Figure 3-1 summarize the health effects observed following oral exposure of animals to di-*n*-butyl phthalate. These effects are discussed below.

3.2.2.1 Death

No studies regarding death in humans following oral exposure to di-*n*-butyl phthalate were located.

Di-*n*-butyl phthalate has low acute toxicity in animals. Single doses of 8,000 mg/kg killed four of nine rats in one study (Smith 1953), but other studies indicate the acute oral LD₅₀ in rats and mice is in excess of 20,000 mg/kg (Hardin et al. 1987; White et al. 1983). The cause of death in these studies was not reported. In mice, an LD₁₀ of 2,500 mg/kg was reported by Hardin et al. (1987).

In a 52-week study in rats, half of the animals given 625 mg/kg/day in feed died during the first week of the study; the cause of death was not determined. No deaths were observed at 125 mg/kg/day (Smith 1953). No deaths were reported in rats or mice that received up to 2,964 or 4,278 mg/kg/day, respectively, in the feed for 13 weeks (NTP 1995). However, pregnant rats may be more susceptible to the lethal effects of di-*n*-butyl phthalate, as 1–2 rats per treatment group died when exposed to 630 to 3000 mg/kg/day for 1 to 9 days during gestation (Ema et al. 1993, 1994; Saillenfait et al. 1998).

The highest NOAEL values and all reliable LOAEL values for death in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.2.2 Systemic Effects

No studies regarding systemic effects in humans following oral exposure to di-*n*-butyl phthalate were located.

Table 3-1. Levels of Significant Exposure to Di-n-butyl phthalate - Oral

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)	
ACUTE EXPOSURE							
Death							
1	Rat (Sprague-Dawley)	1 d (G)				8000 (4/9 died)	Smith 1953
2	Rat	1 d (G)				10000 (2/10 deaths)	White et al. 1983
3	Mouse	8 d 1x/d (G)				2500 (LD ₁₀)	Hardin et al. 1987
4	Mouse	Gd 6-13 1x/d (G)				2500 (5/49 deaths)	Hardin et al. 1987
Systemic							
5	Rat (Sprague-Dawley)	Gd 12-21 1x/d (GO)	Hepatic	500 F			Mylchreest et al. 2000
			Renal	500 F			
			Endocr	500 F			
			Bd Wt	500 F			
6	Rat (Sprague-Dawley)	1 d (G)	Bd Wt	4000	8000	(unspecified decrease in body weight gain)	Smith 1953

Table 3-1. Levels of Significant Exposure to Di-n-butyl phthalate - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	LOAEL		Reference Chemical Form
				NOAEL (mg/kg/day)	Less serious (mg/kg/day)	
Reproductive						
7	Rat (Sprague- Dawley)	4 d 1x/d (G)		500		1000 (decreased testis weight) Cater et al. 1977
8	Rat (Sprague- Dawley)	6 d 1x/d (G)				500 (decreased testis weight) Cater et al. 1977
9	Rat (Wistar)	Gd 0-8 (GO)		1000		1250 (pregnancy rate decreased by 39%) Ema et al. 2000a
10	Rat (Wistar)	7 d (G)				2400 (testicular atrophy) Fukuoka et al. 1989
11	Rat (Wistar)	1 d (G)				2400 (testicular atrophy) Fukuoka et al. 1990
12	Rat (Sprague- Dawley)	9 d 1x/d (GO)				2000 (severe testicular atrophy, decreased testes weight) Gray et al. 1982
13	Rat (Sprague- Dawley)	Gd 12-21 1x/d (GO)		500 F		Mylchreest et al. 2000
14	Rat (Wistar)	7 d (F)				2100 (decrease in testicular weight and number of spermatocytes and spermatogonia) Oishi and Hiraga 1980b

Table 3-1. Levels of Significant Exposure to Di-n-butyl phthalate - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	LOAEL		Reference Chemical Form	
				NOAEL (mg/kg/day)	Less serious (mg/kg/day)		Serious (mg/kg/day)
15	Rat	7 d 1x/d (G)				2400 (histopathological damage, decreased testis weight)	Tanino et al. 1987
16	Mouse (TO)	9 d 1x/d (GO)			2000 (moderate testicular atrophy)		Gray et al. 1982
17	Gn Pig (Dunkin- Harley)	7 d 1x/d (GO)				2000 (severe testicular atrophy, decreased testes weight)	Gray et al. 1982
18	Hamster (Syrian)	9 d 1x/d (GO)		2000			Gray et al. 1982
Developmental							
19	Rat (Wistar)	Gd 7-15 (GO)		500		630 (increased post implantation loss and decreased fetal body weights)	Ema et al. 1993
20	Rat (Wistar)	Gd 7-9 (GO)				750 (increased post implantation loss, decreased fetal body weights, skeletal malformations)	Ema et al. 1994
21	Rat (Wistar)	Gd 10-12 (GO)				750 (increased post implantation loss)	Ema et al. 1994

Table 3-1. Levels of Significant Exposure to Di-*n*-butyl phthalate - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	LOAEL			Reference Chemical Form	
				NOAEL (mg/kg/day)	Less serious (mg/kg/day)	Serious (mg/kg/day)		
22	Rat (Wistar)	Gd 13-15 (GO)				750	(increased post implantation loss; external and skeletal malformations)	Ema et al. 1994
23	Rat (Wistar)	Gd 7-9 (GO)				750	(increased implantation loss per litter, decreased number of live fetuses per litter, decreased fetal body weights, and increased skeletal malformations)	Ema et al. 1995a
24	Rat (Wistar)	Gd 10-12 (GO)				750	(increased implantation loss per litter, decreased number of live fetuses per litter, and decreased fetal body weights)	Ema et al. 1995a
25	Rat (Wistar)	Gd 13-15 (GO)				750	(skeletal and external malformations; increased percent postimplantation loss/litter)	Ema et al. 1995a
26	Rat (Wistar)	Gd 0-11 (F)				895	(complete resorption of litters)	Ema et al. 1997b
27	Rat (Wistar)	Gd 11-21 (F)		331		555	(increase in internal malformations)	Ema et al. 1998

Table 3-1. Levels of Significant Exposure to Di-*n*-butyl phthalate - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less serious (mg/kg/day)	Serious (mg/kg/day)	
28	Rat (Wistar)	Gd 0-8 (GO)		250	500 (decreased fetal body weight)	750 (increased percent postimplantation loss/litter; decreased number of live fetuses/litter; altered sex ratios)	Ema et al. 2000a
29	Rat (Wistar)	Gd 12-14 1x/d (GO)				1000 M (decreased anogenital distance in fetuses)	Ema et al. 2000b
30	Rat (Wistar)	Gd 15-17 1x/d (GO)				500 M (increased incidence of undescended testes; decreased anogenital distance)	Ema et al. 2000b
31	Rat (Wistar)	Gd 18-20 1x/d (GO)				1000 M (decreased anogenital distance in fetuses; 12-15% decrease in fetal body weight)	Ema et al. 2000b
32	Rat (Sprague- Dawley)	Gd 14-Ld 3 1x/d (GO)				500 (reduced AGD, increased number of retained nipples, decreased androgen-dependent tissue weights in F1 males)	Gray et al. 1999

Table 3-1. Levels of Significant Exposure to Di-n-butyl phthalate - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/duration/frequency (Specific route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less serious (mg/kg/day)	Serious (mg/kg/day)	
33	Rat (Long-Evans)	Gd 16-19 1x/d (GO)				500 (reduced AGD, increased number of retained nipples, decreased androgen-dependent tissue weights in F1 males)	Gray et al. 1999
34	Rat (Sprague-Dawley)	Gd 12-21 1x/d (GO)			100 M (delayed preputial separation)	250 M (malformations of the epididymis; decreased AGD; retained nipples)	Mylchreest et al. 1999
35	Rat (Sprague-Dawley)	Gd 12-21 1x/d (GO)		50 ^b M	100 M (retained areolas or nipples)	500 M (decreased anogenital distance; small sex accessory glands; decreased testes weight; malformations of reproductive tract)	Mylchreest et al. 2000
36	Rat (Sprague-Dawley)	Gd 14 (GO)		500	1000 (increased incidence of accessory 14th rib)	2000 (increased incidence of fused sternbrae and reproductive system anomalies; decreased percentage of male fetuses)	Saillenfait et al. 1998

Table 3-1. Levels of Significant Exposure to Di-*n*-butyl 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)	
INTERMEDIATE EXPOSURE							
Systemic							
37	Rat (Wistar)	34-36 d (F)	Hepatic		470	(liver necrosis; decreased mitochondrial enzyme activities)	Murakami et al. 1986a
			Bd Wt		470	(15% decrease in body weight gain)	

Table 3-1. Levels of Significant Exposure to Di-n-butyl phthalate - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	LOAEL		Reference Chemical Form	
				NOAEL (mg/kg/day)	Less serious (mg/kg/day)		Serious (mg/kg/day)
38	Rat (Fischer- 344)	13 wk (F)	Resp	2964			NTP 1995
			Cardio	2964			
			Gastro	2964			
			Hemato	176	359 M (anemia)		
			Hepatic	176	359 (increased liver weight; increased palmitoyl-CoA oxidase activity)		
			Hepatic		176 (decreased serum triglyceride levels)		
			Renal	176	359 M (increased relative kidney weight)		
			Endocr	720	1540 M (decreased serum testosterone concentration)		
Bd Wt	359	720 (8-11% decrease in body weight gain)	2964 (emaciation)				

Table 3-1. Levels of Significant Exposure to Di-n-butyl phthalate - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	LOAEL		Reference Chemical Form	
				NOAEL (mg/kg/day)	Less serious (mg/kg/day)		Serious (mg/kg/day)
39	Rat (Wistar)	3 mo 1x/d (F)	Hemato	152	752 M	(decreased erythrocyte, hemoglobin, and hematocrit levels)	Schilling et al. 1992
			Hepatic	152	752	(decreased lipid deposition in liver and blood triglyceride levels; increased glucose and albumin levels)	
			Endocr	152	752	(decreased triiodothyronine levels)	
40	Mouse (B6C3F1)	13 wk (F)	Resp	3689			NTP 1995
			Cardio	3689			
			Gastro	3689			
			Hemato	1601	3689	(decreased hematocrit)	
			Hepatic	812	1601	(cytoplasmic alterations)	
			Renal	3689			
			Endocr	3689			
Bd Wt	353	812	(10% decrease in body weight gain)				

Table 3-1. Levels of Significant Exposure to Di-n-butyl phthalate - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less serious (mg/kg/day)	Serious (mg/kg/day)	
Neurological							
41	Rat (Wistar)	Daily 3 mo (F)		752			Schilling et al. 1992
Reproductive							
42	Rat (Long- Evans)	weaning-Ld 20 1x/d (GO)			250 M (delayed preputial separation in the P0 generation)	500 (reduced fertility in P0 males and females; testicular atrophy, reduced sperm production in males; abortion in females)	Gray et al. 1999
43	Rat COBS CD	Daily 110 d (F)		500			IRDC 1984
44	Rat COBS CD	Daily 103 d (F)		500			IRDC 1984
45	Rat	34-36 d (F)		470		4700 (decreased testicular weight; marked spermatogenic damage)	Murakami et al. 1986a

Table 3-1. Levels of Significant Exposure to Di-n-butyl phthalate - Oral (continued)

Key to figure ^a	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	LOAEL		Reference Chemical Form
				NOAEL (mg/kg/day)	Less serious (mg/kg/day)	
46	Rat (Fischer- 344)	Gd 1-21 (F)			950 F (shortening of gestation length)	NTP 1995
47	Rat (Fischer- 344)	13 wk (F)		359° M 2943 F	720 M (degeneration of germinal epithelium in seminiferous tubules)	1540 M (hypospermia of the epididymis) NTP 1995
48	Rat (Sprague- Dawley)	26 wk (F)		256		509 (decreases in mating, pregnancy, and fertility indices) NTP 1995
49	Rat (Wistar)	Daily 15 d (GO)				250 (testicular damage and defective spermatogenesis) Srivastava et al. 1990
50	Mouse (B6C3F1)	13 wk (F)		3689° M 4278 F		NTP 1995
51	Mouse (Swiss CD-1)	18 wk (F)		580		1950 (decreases in fertility index) NTP 1995
Developmental						
52	Rat (Long- Evans)	weaning-Ld 20 1x/d (GO)				250 (malformations and reduced fecundity in F1 males and females) Gray et al. 1999

Table 3-1. Levels of Significant Exposure to Di-n-butyl 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)	
53	Rat COBS CD	103 d 1x/d (F)				500 (testicular degeneration in offspring)	IRDC 1984
54	Rat (Sprague- Dawley)	Daily Gd 3-21, Ld 2-20 (GO)				250 (degeneration and atrophy of seminiferous tubules)	Mylchreest et al. 1998b
55	Rat (Fischer-344)	Gd1-21, Ld1-28, Pd28-56 (F)		120	240 (5% decrease in pup body weight) 480 M (hypospermia in offspring)	960 (decreased pup survival)	NTP 1995
56	Rat (Fischer-344)	Gd1-21, Ld1-28 (F)				950 (decreased number of pups/litter and pup body weight)	NTP 1995
57	Rat (Fischer-344)	Gd1-21, Ld1-28, 13 wk after weaning (F)		279		571 M (testicular atrophy)	NTP 1995

Table 3-1. Levels of Significant Exposure to Di-*n*-butyl phthalate - Oral (continued)

Key to figure	Species (Strain)	Exposure/duration/frequency (Specific route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less serious (mg/kg/day)	Serious (mg/kg/day)	
58	Rat (Sprague-Dawley)	26 wk (F)		256		509 (decreased epididymal, cauda epididymal, testes, seminal vesicle, prostate, and ovary wt, testicular degeneration and hypospermia)	NTP 1995
						80 (decreased number of live pups per litter)	
59	Mouse (B6C3F1)	Gd1-21, Ld1-28, Pd 28-56 (F)		920		1380 (decreased pup survival)	NTP 1995
				230	460 (decreased body weight after 4 weeks post weanling exposure)		

Table 3-1. Levels of Significant Exposure to Di-n-butyl phthalate - Oral (continued)

Key to ^a figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	LOAEL			Reference Chemical Form
				NOAEL (mg/kg/day)	Less serious (mg/kg/day)	Serious (mg/kg/day)	
60	Mouse (Swiss CD-1)	18 wk (F)		580		1950 (decreases in average number of litters per breeding pair and live pups per litter)	NTP 1995

^aThe number corresponds to entries in Figure 3-1.

^bUsed to derive an acute oral minimal risk level (MRL) of 0.5 mg/kg-day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

^cDifferences in levels of health effects and cancer effects between males and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; (F) = feed; F = female; (G) = gavage; gastro = gastrointestinal; Gd = gestation day; (GO) = gavage in oil; Hemato = hematological; LD₁₀ = lethal dose, 10% kill; Ld = lactation day; LOAEL = lowest-observed-adverse-effect level; M = male; mg/kg/day = milligram per kilogram per day; mo = month(s); NOAEL = no-observed-adverse-effect level; Pd = post-natal day; Resp = respiratory; wk = week(s); x = time

Figure 3-1. Levels of Significant Exposure to Di-*n*-butyl phthalate - Oral
Acute (≤ 14 days)

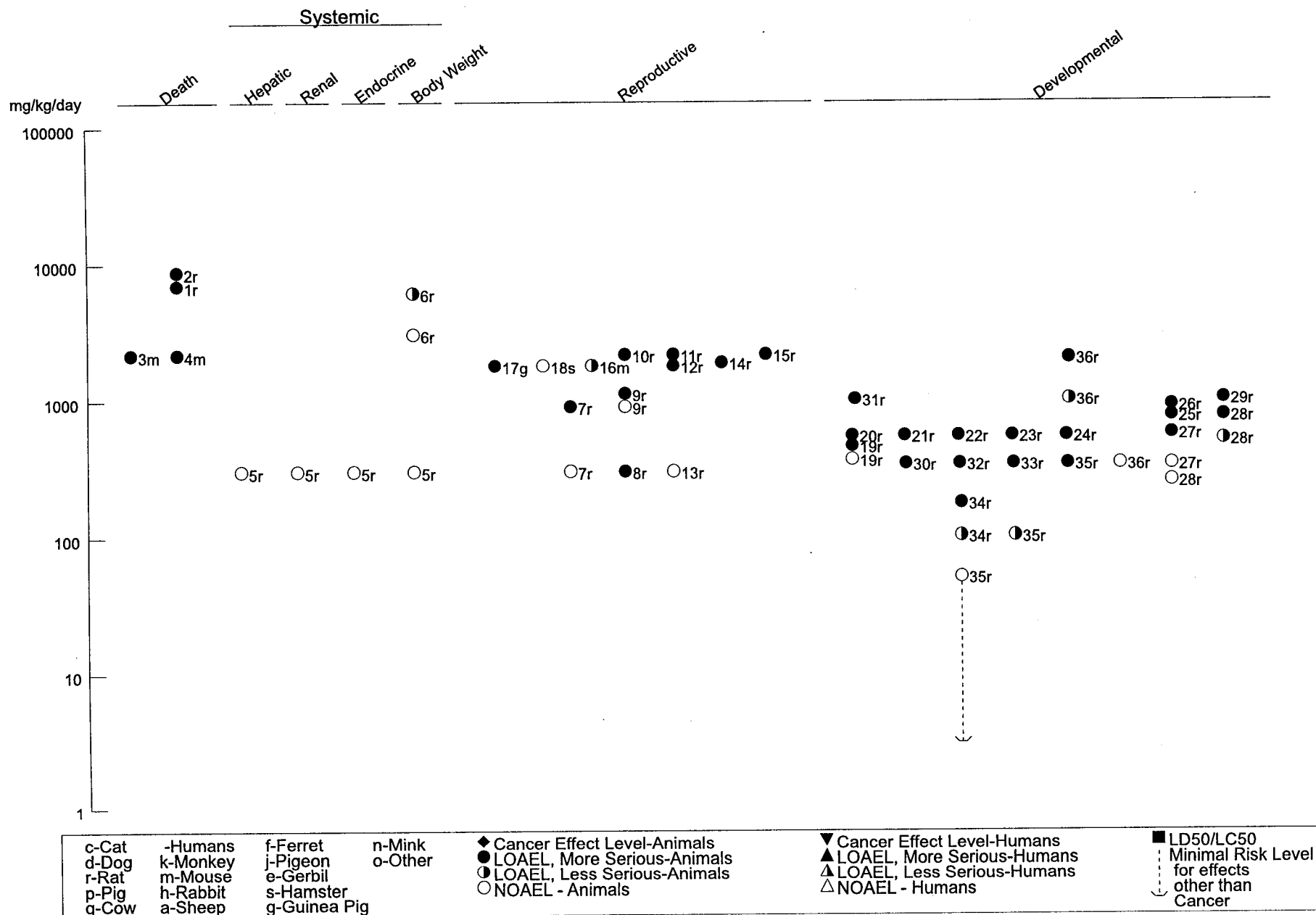
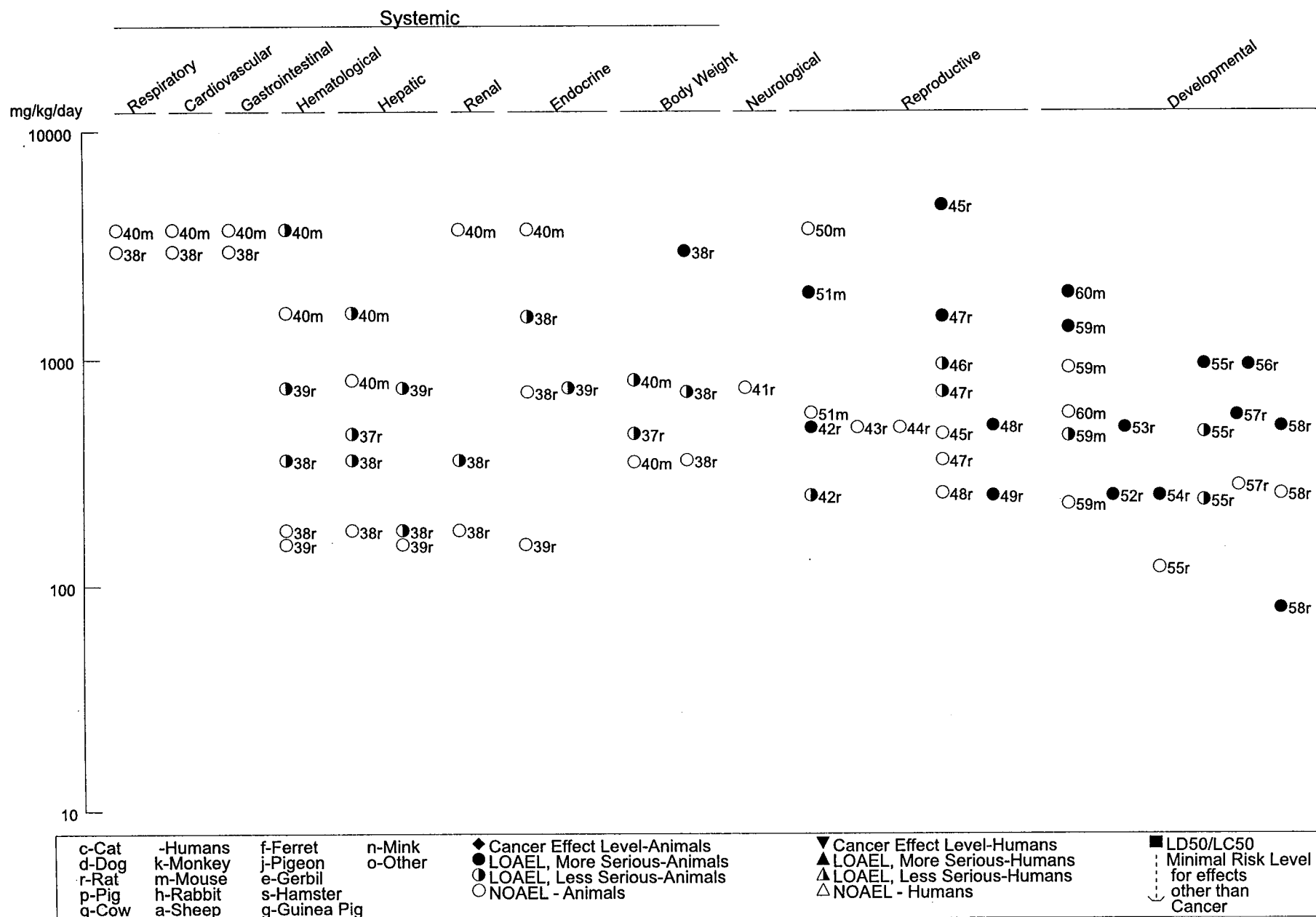


Figure 3-1. Levels of Significant Exposure to Di-*n*-butyl phthalate - Oral (continued)

Intermediate (15-364 days)



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Respiratory Effects. No histological alterations were observed in the respiratory tract tissues of rats and mice exposed to up to 2,964 and 4,278 mg/kg/day, respectively, di-*n*-butyl phthalate in the diet for 13 weeks (NTP 1995).

Cardiovascular Effects. No histological alterations were observed in the heart of rats and mice exposed to up to 2,964 and 4,278 mg/kg/day, respectively, di-*n*-butyl phthalate in the diet for 13 weeks (NTP 1995).

Gastrointestinal Effects. No histological alterations were observed in the gastrointestinal tract tissues of rats and mice exposed to up to 2,964 and 4,278 mg/kg/day, respectively, di-*n*-butyl phthalate in the diet for 13 weeks (NTP 1995).

Hematological Effects. Minimally severe anemia was observed in male rats exposed to 369 mg/kg/day di-*n*-butyl phthalate in the diet for 13 weeks (NTP 1995). The study authors noted that hemoconcentration by dehydration (as evidenced by higher albumin concentrations) may have masked the effects, and that the anemia may have been more severe than the data indicated. No hematological effects were observed in the female rats. Decreases in erythrocyte, hemoglobin, and hematocrit levels were also observed in male rats exposed to 752 mg/kg/day in the diet (Schilling et al. 1992) and a decrease in hematocrit was observed in female mice exposed to 2,137 mg/kg/day di-*n*-butyl phthalate in the diet (NTP 1995).

Biochemical parameters and histopathological evaluation of the spleen of rats showed no effects at doses up to 1,200 mg/kg/day (Nikonorow et al. 1973; Smith 1953). Increased absolute and relative spleen weight was observed in rats at a dose of 2,500 mg/kg/day (Murakami et al. 1986a, 1986b), but without additional information on histopathological changes and evaluation of hematological parameters, the significance of this isolated finding cannot be determined.

Musculoskeletal Effects. No studies regarding musculoskeletal effects in animals following oral exposure to di-*n*-butyl phthalate were located.

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Hepatic Effects. In animals, minimal effects on the liver were observed after acute exposure to di-*n*-butyl phthalate. Increased absolute liver weight was observed in rats and mice given di-*n*-butyl phthalate at 2% in the diet (1,000–2,600 mg/kg/day) for 7 days (Oishi and Hiraga 1980a, 1980b) or in rats and mice that received at least 359 or 2137 mg/kg/day, respectively, in the diet for 13 weeks (NTP 1995). Increased relative liver weight in animals was observed in several studies with di-*n*-butyl phthalate at doses of 348 mg/kg/day and higher for 21 days or more (Bell 1982; BIBRA 1986; Murakami et al. 1986a, 1986b; Nikonorow et al. 1973). In these studies, the increases in relative liver weight may simply reflect body weight decreases caused by di-*n*-butyl phthalate in those animals.

Slight, but statistically significant, increases in microsomal enzyme activity levels were observed in the livers of rats given di-*n*-butyl phthalate by gavage for 5 days at doses of 2.8 and 27.8 mg/kg/day, but not at 278 mg/kg/day (Walseth and Nilsen 1986). The authors considered di-*n*-butyl phthalate to be a weak inducer of microsomal enzymes. Increased microsomal enzyme activity was observed in the livers of rats exposed to 1,000 mg/kg/day in the diet for 7 days (Kawashima et al. 1983).

Longer exposure to di-*n*-butyl phthalate was found to interfere with mitochondrial respiration. Mitochondrial respiration was inhibited in rats fed di-*n*-butyl phthalate at 2,500 mg/kg/day for 35 days (Murakami et al. 1986b). Evaluation of liver tissue by electron microscopy revealed an increase in the number of mitochondria, suggesting that the organ is compensating for the inhibitory effects of the di-*n*-butyl phthalate on mitochondrial function (Murakami et al. 1986a). NTP (1995) reported hepatocellular cytoplasmic alterations, consistent with glycogen depletion, in rats and mice exposed to 720 and 1601 mg/kg/day, respectively, di-*n*-butyl phthalate in the diet for 13 weeks. Liver necrosis was noted at doses of 470 mg/kg/day and higher, an effect possibly related to the effects of di-*n*-butyl phthalate on liver mitochondria (Murakami et al. 1986a). Other studies using higher doses have found no liver necrosis (BIBRA 1986; Nikonorow et al. 1973; NTP 1995). No explanation for the discrepant results is evident.

Decreases in serum triglyceride and cholesterol levels were observed in rats exposed to dietary di-*n*-butyl phthalate for 13 weeks (NTP 1995). Although the mechanism for these alterations is not known, this finding may be associated with peroxisome proliferation.

Proliferation of peroxisomes and increases in peroxisomal enzymes have been reported in rat liver cells by several investigators (BIBRA 1986; Murakami et al. 1986a) at doses of 2,131 mg/kg/day for 21 days or more. In rats exposed for 13 weeks, induction of peroxisomal enzyme activity (acyl CoA oxidase) was

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observed at doses of 353 mg/kg/day, but not 176 mg/kg/day (NTP 1995). This response may contribute to the increase in liver weight discussed above, especially in males (Murakami et al. 1986a, 1986b).

Renal Effects. Oral exposure to di-*n*-butyl phthalate has been reported to cause decreased kidney weight after 7 days of exposure of mice to 2,600 mg/kg/day (Oishi and Hiraga 1980a) and increased kidney weight after 21 days of exposure of rats to 1,248 mg/kg/day (BIBRA 1986). Relative kidney weights were increased in male and female rats administered 359 and 712 mg/kg/day, respectively, for 13 weeks, and absolute and relative kidney weights were increased in female mice exposed to 238 mg/kg/day for 13 weeks (NTP 1995); decreased absolute kidney weights in male and female rats and male mice were considered secondary to body weight changes. No histopathologic lesions of the kidney have been observed in rats exposed to di-*n*-butyl phthalate (BIBRA 1986; Nikonorow et al. 1973; NTP 1995).

Endocrine Effects. No histological alterations in endocrine tissue have been observed in rats and mice exposed to up to 2,964 and 4,278 mg/kg/day, respectively, di-*n*-butyl phthalate in the diet for 13 weeks (NTP 1995). Phthalates are a class of chemicals that have been implicated as having estrogenic properties. There is *in vitro* evidence of the weak estrogenic behavior of di-*n*-butyl phthalate; however, *in vivo* results do not support these findings. Studies in rats indicate that di-*n*-butyl phthalate has anti-androgenic properties (Ema et al. 1998, 2000b; Gray et al. 1999; Mylchreest et al. 1999, 2000). These are discussed in Section 3.6 Endocrine Disruption.

Dermal Effects. No studies regarding dermal effects in humans or animals following oral exposure to di-*n*-butyl phthalate were located.

Ocular Effects. No studies regarding ocular effects in humans following oral exposure to di-*n*-butyl phthalate were located.

No changes to the refracting media of the eye were noted in Wistar rats receiving 1,075 (male) or 1,111 (female) mg/kg/day di-*n*-butyl phthalate in the feed for 3 months (Schilling et al. 1992).

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Body Weight Effects. Several studies have evaluated the effect of oral exposure of animals to di-*n*-butyl phthalate on body weight (BIBRA 1986; Gray et al. 1982; Lamb et al. 1987; Murakami et al. 1986, 1986b; Nikonorow et al. 1973; NTP 1984, 1995; Oishi and Hiraga 1980a; Smith 1953). Decreases in body weight gain were only observed at higher doses (8,000 mg/kg/day) in the acute-duration studies (Gray et al. 1982; Smith 1953). Decreases in body weight gain have been observed at lower doses following repeated exposure: 250 mg/kg/day in rats exposed for 34–36 days (Murakami et al. 1986a), 720 mg/kg/day in rats for 13 weeks (NTP 1995), and 353 mg/kg/day in mice exposed for 13 weeks (NTP 1995).

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-1 and Figure 3-1.

Metabolic Effects. No studies regarding metabolic effects in humans or animals following oral exposure to di-*n*-butyl phthalate were located.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies regarding immunological effects in humans or animals following oral exposure to di-*n*-butyl phthalate were located.

3.2.2.4 Neurological Effects

No studies regarding neurological effects in humans following oral exposure to di-*n*-butyl phthalate were located.

Male and female Wistar rats exposed to 1,075 and 1,111 mg/kg/day di-*n*-butyl phthalate, respectively, in the feed for 3 months showed no signs of neurological impairment, as assessed by a functional observational battery of sensory and motor function parameters (Schilling et al. 1992). No gross or histological lesions of the brain were noted in rats or mice exposed to up to 2,964 or 4,000 mg/kg/day, respectively, for 13 weeks, or exposed throughout gestation, lactation, and then for 13 weeks.

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3.2.2.5 Reproductive Effects

A weak, negative correlation was reported between sperm density and di-*n*-butyl phthalate concentration in semen from male university students (Murature et al. 1987). However, lack of data and inadequate data analysis in this study prevent the establishment of a causal relationship between sperm density and di-*n*-butyl phthalate. No other studies regarding reproductive effects in humans following oral exposure to di-*n*-butyl phthalate were located.

Oral exposure to di-*n*-butyl phthalate had adverse effects on the male reproductive system in several animal species (rats, mice, and guinea pigs). Testicular atrophy (including decreases in testicular weight, decreases in spermatocytes and spermatogonia, and sloughing of germ cells) have been observed in Sprague-Dawley or Wistar rats receiving 1,000 mg/kg/day or higher for 4 or 5 days (Cater et al. 1977; Gray and Gangolli 1986), 2,400 mg/kg/day for 1 or 7 days via gavage (Fukuoka et al. 1989, 1990), or 2,100 mg/kg/day in the diet for 7 days (Oishi and Hiragi 1980b). Gray et al. (1999) found that Long-Evans hooded rats exposed to 250 mg/kg/day from weaning through puberty and young adulthood also showed testicular atrophy and reduced sperm production. Fukuoka et al. (1990) observed sloughing of the germ cells in the seminiferous tubules 6 hours after dosing with di-*n*-butyl phthalate. Three to seven days after a single gavage dose was administered, more severe damage including dissociation of mature germ cells (i.e., spermatozoa, spermatids, and spermatocytes) from seminiferous tubule germinal epithelium was observed (Fukuoka et al. 1990).

Srivastava et al. (1990) found a minimal amount of seminiferous tubular damage (approximately 5% of tubules affected) in Wistar rats following gavage administration of 250 mg/kg/day in groundnut oil for 15 days, more moderate tubular damage (20% affected) with decreased testicular weight at 500 mg/kg/day, and marked degeneration of seminiferous tubules at 1,000 mg/kg/day. Similarly, Gray et al. (1999) found testicular atrophy, with accompanying decreased sperm production, in Long-Evans rats following gavage administration of 500, but not 250 mg/kg/day, di-*n*-butyl phthalate in corn oil from weaning into adulthood; no histological data were provided. In contrast, NTP (1995) did not find testicular alterations in rats exposed to 359 mg/kg/day in the diet for 13 weeks. Testicular atrophy was observed at 720 mg/kg/day and hypospermia at 1,540 mg/kg/day. The different routes of exposure (gavage versus diet) may explain the different results in these studies.

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The testicular effects of acute exposure of rats to di-*n*-butyl phthalate appear to be at least in part reversible. Tanino et al. (1987) showed that 2 weeks after discontinuation of the administration of di-*n*-butyl phthalate (2,400 mg/kg/day for 7 days), some regeneration of seminiferous tubules had occurred. Three weeks after treatment, active spermatogenesis was observed in almost all tubules. However, vacuolation of germinal epithelium and decreased number of sperm were still evident.

Species and sex differences in the reproductive toxicity of di-*n*-butyl phthalate are evident. While severe seminiferous tubular atrophy was observed in rats and guinea pigs at 2,000 mg/kg/day for 7–9 days, only focal atrophy was reported in mice at the same dose, and no effects on the testes were seen in Syrian hamsters (Gray et al. 1982). Testicular atrophy was observed in rats following intermediate-duration exposure to 720 mg/kg/day (NTP 1995); this effect was not observed in mice exposed to 3,689 mg/kg/day (NTP 1995). The species differences may be related to the greater ability of some species to conjugate the primary metabolite of di-*n*-butyl phthalate (see Section 3.3.3.2).

No histopathological alterations in reproductive tissues or effects on estrus cycling were observed in female rats following a 13-week exposure to doses as high as 2,943 mg/kg/day (NTP 1995), and no statistically significant differences from controls in number of implantation sites or live pups per litter were seen in Sprague-Dawley rats exposed to up to 500 mg/kg/day on gestational days 12–21 (Mylchreest et al. 2000). However, pregnancy rate (nonpregnancy=no detectable implantation sites) was decreased in female Wistar rats (bred to untreated males) receiving 1,250 or 1,500 mg/kg/day di-*n*-butyl phthalate via gavage administration on gestational days 0–8, and preimplantation losses were increased at 1,500 mg/kg/day (Ema et al. 2000a). Also, a cross-over mating study (NTP 1995) in mice suggests that di-*n*-butyl phthalate may impair fertility in exposed females. Decreases in fertility and average number of litters per breeding pair and live pups per litter were observed in female mice exposed to 1,950 mg/kg/day and mated with control males.

In a three-generation study in Long-Evans rats, exposure of the first generation (F₀) males to 250 mg/kg/day di-*n*-butyl phthalate via gavage administration from weaning through early adulthood statistically significantly delayed preputial separation (an index of puberty) in a dose-dependent manner (39.5, 42.6, 43.4, and 44.4 days in the 0, 250, 500, and 1,000 mg/kg/day groups, respectively) (Gray et al. 1999). In the same study, exposure to 500 mg/kg/day resulted in decreased fertility in (F₀) males and females (bred to untreated rats), and caused mid-gestation abortions in females bred to untreated males (Gray et al. 1999). Infertility in males was related to testicular atrophy and reduced sperm production.

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Reproductive malformations in second generation (F₁) male pups (exposed to di-*n*-butyl phthalate only *in utero* and via lactation) included testicular nondescent and hypospadias. Reduced fecundity was seen in similarly treated (250 or 500 mg/kg/day) (F₁) mating pairs under continuous breeding conditions, which may have been caused, at least in part, by reduced cauda epididymal sperm counts in (F₁) males.

The highest NOAEL and all reliable LOAEL values for reproductive effects are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans following oral exposure to di-*n*-butyl phthalate.

A number of studies have examined the developmental toxicity of di-*n*-butyl phthalate in animals following acute- and intermediate-duration oral exposure (Ema et al. 1993, 1994, 1995a, 1997a, 1997b, 1998, 2000a, 2000b; Gray et al. 1999; Hardin 1987; IRDC 1984; Mylchreest et al. 1998b, 1999, 2000, NTP 1995; Saillenfait et al. 1998). The observed developmental effects include increases in postimplantation losses, decreases in the number of live fetuses per litter, decreases in fetal/pup body weights, increases in incidences of external, skeletal and internal malformations, and altered reproductive development in the offspring. A series of gavage-administration studies, conducted by Ema and associates, reported postimplantation losses and decreases in the number of live fetuses in Wistar rats receiving gavage doses of 630 mg/kg/day on gestational days 7–15 (Ema et al. 1993) or 750 mg/kg/day on gestational days 0–8 (Ema et al. 2000a), 7–9 (Ema et al. 1994, 1995a), 10–12 (Ema et al. 1994, 1995a), or 13–15 (Ema et al. 1994); no losses were reported after dosing with 500 mg/kg/day on gestational days 7–15 (Ema et al. 1993). In a series of single gavage dose studies by Ema et al. (1997a), postimplantation losses were observed in rats dosed with 1,500 mg/kg/day di-*n*-butyl phthalate on gestational days 6, 8, 9, 10, 12, 13, 14, 15, and 16. At doses of 750 mg/kg/day (administered on gestational days 7–15), >80% of the litters were totally resorbed; decreases in maternal body weight and food consumption were also observed at this dosage (Ema et al. 1993). Approximately 10–20% of the litters were completely resorbed when the dams were dosed with 750 mg/kg/day for 3 days during gestation (between gestational days 7 and 17) (Ema et al. 1994, 1995a); 20–100% were totally resorbed at 1,500 mg/kg/day (Ema et al. 1994, 2000b); no litters were totally resorbed following exposure to up to 1,500 mg/kg/day on gestational days 18–20 (Ema et al. 2000b). Maternal food consumption and body

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weight gain were decreased at 500 mg/kg/day or greater (Ema et al., 2000b). Hardin et al. (1987) also reported fetal losses (no viable litters) in mice receiving gavage doses of 2,500 mg/kg/day on gestational days 6–13. A pilot study conducted by Saillenfait et al. (1998) also found postimplantation losses in Sprague-Dawley rats receiving single gavage doses of 2,000 mg/kg/day on gestational days 13, 14, or 15 (loss per litter of 23.04, 64.46, or 37.55%, respectively). Increased pup losses have also been observed in intermediate-duration studies. Female Long-Evans rats treated with 500 mg/kg/day di-*n*-butyl phthalate from weaning through puberty and gestation aborted their litters around mid-gestation (Gray et al. 1999). Rats exposed to 250 or 500 mg/kg/day di-*n*-butyl phthalate *in utero* and via lactation produced fewer pups than controls (means of 108, 78, and 59 pups/breeding pair over 11 breeding cycles for control, 250, and 500 mg/kg/day, respectively) when mated with similarly treated rats under continuous breeding conditions (Gray et al. 1999). A decrease in the number of live pups per litter was observed in rats exposed via the diet to 950 mg/kg/day on gestational days 1–21 (NTP 1995). Decreases in the number of live pups per litter were also observed in continuous breeding studies conducted for NTP (1995) in which rats and mice were exposed to 80 and 1,950 mg/kg/day, respectively, di-*n*-butyl phthalate in the diet for 112 or 98 days, respectively. A NOAEL for this effect was not identified in the rat study; the mouse study identified a NOAEL of 580 mg/kg/day. Another developmental toxicity study conducted for NTP (1995) reported decreased survival in the pups of rats and mice exposed to di-*n*-butyl phthalate in the diet throughout gestation, lactation, and postnatal days 28–56. The LOAELs identified in the rat and mouse studies were 960 and 1,380 mg/kg/day, respectively; the NOAELs for pup survival were 720 and 920 mg/kg/day, respectively.

Decreased body weights have been observed in the offspring of rats following acute- or intermediate-duration exposure (Ema et al. 1993, 1994, 1995a, 1997a, 2000a, 2000b; NTP 1995). Dose-related decreases in fetal body weights were observed in rats receiving gavage doses of 500 mg/kg/day or higher di-*n*-butyl phthalate on gestational days 0–8 (Ema et al. 2000a) or 630 mg/kg/day or higher on gestational days 7–15 (Ema et al. 1993). Decreases in body weights were also observed in the fetuses of rats receiving gavage doses of 750 mg/kg/day on gestational days 7–9, 10–12, or 13–15 (Ema et al. 1994, 1995a), 1,000 mg/kg/day on gestational days 12–14 or 18–20 (Ema et al. 2000b), or 1,500 mg/kg/day on gestational days 6, 7, 8, 9, 10, 11, 16, or 15–17 (Ema et al. 1997a, 2000b). Body weight was not affected when the dams were exposed on gestational days 12, 13, 14, or 15. Similarly, rat pups exposed *in utero* to up to 500 mg/kg/day on gestational days 12–21 had no significant decrease in body weight (Mylchreest et al. 2000). Several of the NTP (1995) developmental toxicity studies found decreases in pup body weight. The identified LOAELs were 240 mg/kg/day (NOAEL of 120 mg/kg/day) in rats exposed

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throughout gestation, lactation, and postnatal days 28–56, 950 mg/kg/day in rats exposed throughout gestation and lactation, and in mice exposed to 460 mg/kg/day (NOAEL of 230 mg/kg/day) throughout gestation, lactation, and postnatal days 28–56.

External and skeletal malformations have been observed in the offspring of rats exposed to di-*n*-butyl phthalate for acute durations (Ema et al. 1993, 1994, 1995, 1997a; Saillenfait et al. 1998). Increases in the incidence of skeletal and/or external malformation have been observed in a number of gavage administration studies conducted by Ema and associates. The skeletal effects, primarily fusion or absence of cervical vertebral arches, were observed in the fetuses of rats exposed to 750 mg/kg/day on gestational days 7–9 or 13–15 (Ema et al. 1994, 1995a). External malformations (cleft palate) were observed in fetuses of rats receiving 750 mg/kg/day gavage doses of di-*n*-butyl phthalate on gestational days 13–15, but not on days 7–9 or 10–12 (Ema et al. 1994, 1995a). Saillenfait et al. (1998) also found increases in the occurrence of skeletal variations (accessory 14th rib) and malformations (fused sternbrae) in the fetuses of rats receiving a single gavage dose of 1,000 or 2,000 mg/kg/day, respectively, on gestational day 14. A serial study conducted by Ema et al. (1997a) was designed to identify the critical periods for skeletal and external malformations in rats receiving a single gavage dose of 1,500 mg/kg/day on one of gestational days 6–16; maternal toxicity (death or decreases in adjusted body weight gain) was not observed in this study. Increases in external malformations were only observed in the fetuses exposed on gestational day 15 and skeletal malformations were observed in fetuses exposed on gestational days 8, 9, or 15.

Acute- and intermediate-duration studies have reported reproductive effects in rat offspring (Ema et al. 1998, 2000a, 2000b; Gray et al. 1999; IRDC 1984; Mylchreest et al. 1998b, 1999, 2000; NTP 1995). Rats exposed to 500 mg/kg/day di-*n*-butyl phthalate on gestational days 12–21 exhibited a number of developmental reproductive abnormalities, including retained nipple buds or areolas, decreased anogenital distance and anogenital distance/body weight ratio, absent or malformed epididymis, absent or malformed vas deferens, and hypospadias (Mylchreest et al. 2000). Only retained nipple buds/areolas were seen in rats exposed to 100 mg/kg/day, and no developmental effects were seen at 50 mg/kg/day or less. This study was used to derive an acute-duration oral MRL for di-*n*-butyl phthalate. In an acute-duration study by Ema et al. (1998), an increased incidence of undescended testes (an internal malformation) and decreased anogenital distance and anogenital distance/body weight ratio were observed in the fetuses of rats receiving gavage doses of 555 or 661 mg/kg/day on gestational days 11–21; no effects were observed at 331 mg/kg/day. Effects seen in other acute-duration studies involving gestational or gestational/

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lactational exposure to 250–1,500 mg/kg/day included retained nipple buds or areolas, decreased anogenital distance and anogenital distance/body weight ratio, absent or malformed epididymis, degeneration of seminiferous epithelium, absent or malformed vas deferens, decreased androgen-dependent tissue weights (ventral prostate, epididymis, cauda epididymis, testes, glans penis, and levator ani-bulbocavernosus), hypospadias, increased incidence of cryptorchidism, and delayed preputial separation (Ema et al. 2000a, 2000b; Gray et al. 1999; Mylchreest et al. 1999). The reproductive effects observed in the intermediate-duration studies are similar to those observed in adult rats orally exposed to di-*n*-butyl phthalate, including degeneration and atrophy of the seminiferous tubules and hypospermia. Degeneration and atrophy of the seminiferous tubules were observed in the offspring of rats receiving daily gavage doses of 250 mg/kg/day on gestational days 3–21 and lactational days 2–20 (Mylchreest et al. 1998b). Male offspring of rat dams exposed to 250 mg/kg/day di-*n*-butyl phthalate or higher from weaning through mating, gestation, and lactation showed reduced cauda epididymal sperm counts and increased incidence of urogenital malformations (Gray et al. 1999). Female offspring in the same study (Gray et al. 1999) showed increased incidence of uterine abnormalities (partial agenesis or lack of implants in one uterine horn). Two studies conducted for NTP (1995) and a study by IRDC (1984) examined reproductive organs of F₁ rats exposed to di-*n*-butyl phthalate *in utero*, during lactation, and for 7 (IRDC 1984), 10 (NTP 1995), or 13 weeks postweaning. IRDC (1984) and NTP (1995) reported testicular degeneration/atrophy in the F₁ rats exposed to 500 (IRDC 1984) or 571 (NTP 1995) mg/kg/day; this effect was not observed in offspring of rats only exposed *in utero* and during lactation (IRDC 1984). Decreases in epididymal, cauda epididymal, testes, seminal vesicle, prostate gland, and ovary weights, testicular degeneration, and hypospermia were observed in the F₁ rats exposed to 509 mg/kg/day *in utero*, during lactation, and through delivery of the F₂ generation (10 weeks) (NTP 1995).

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.2.7 Cancer

No studies regarding carcinogenic effects of di-*n*-butyl phthalate in humans after oral exposure to di-*n*-butyl phthalate were located. Rats exposed for 15–21 months to doses of 100–500 mg/kg/day were reported not to develop cancer, but no details of the study or the examination for tumors were provided

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(Krauskopf 1973). No other studies on the carcinogenic effects of chronic ingestion of di-*n*-butyl phthalate were located.

3.2.3 Dermal Exposure

Available data on the effects of dermal exposure to di-*n*-butyl phthalate are presented in Table 3-2. These studies are discussed below.

3.2.3.1 Death

No studies regarding death in humans following dermal exposure to di-*n*-butyl phthalate were located.

The subchronic (90-day) dermal LD₅₀ in rabbits has been reported to be >4,200 mg/kg/day (Lehman 1955).

3.2.3.2 Systemic Effects

No studies regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, ocular, metabolic, or body weight effects in humans or animals following dermal exposure to di-*n*-butyl phthalate were located.

Renal Effects. No information concerning renal effects in humans following dermal exposure to di-*n*-butyl phthalate was located. Histological evidence of slight kidney damage was noted in rabbits after 90 days of dermal application of 4,200 mg/kg/day (Lehman 1955). No details about the study or specifics about the type of kidney damage were given. In this study, a NOAEL of 2,100 mg/kg/day was identified.

Dermal Effects. Some cosmetic preparations containing di-*n*-butyl phthalate cause slight irritation to human skin (Cosmetic Ingredient Review Panel 1985). A single dermal application of 520 mg/kg/day of di-*n*-butyl phthalate was reported to be slightly irritating to skin and "quite irritating" to mucous membranes of rabbits (Lehman 1955). In a 90-day study, doses up to 4,200 mg/kg/day were described as slightly irritating, and slight dermatitis was reported. No data were presented, and the no-effect level was not given.

Table 3-2. Levels of Significant Exposure to Di-n-butyl phthalate - Dermal

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
				Less serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE						
Systemic						
Rabbit	1x (F)	Dermal/Oc		520	(slightly irritated)	Lehman 1955
INTERMEDIATE EXPOSURE						
Death						
Rabbit	90d 1x/d				4200 (LD ₅₀)	Lehman 1955
Systemic						
Rabbit	90d 1x/d	Renal	2100	4200	(kidney damage)	Lehman 1955

d = day(s); (F) = feed; LD₅₀ = lethal dose, 50%; LOAEL = lowest-observed-adverse-effect level; mg/kg/day = milligram per kilogram per day; NOAEL = no-observed-adverse-effect level; Oc = ocular; x = time

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3.2.3.3 Immunological and Lymphoreticular Effects

Di-*n*-butyl phthalate does not appear to be a skin sensitizer. A variety of cosmetic materials (e.g., deodorants, nail polish) containing 4.5–9% di-*n*-butyl phthalate were not skin sensitizers when tested on 50–250 individuals per sample (Cosmetic Ingredient Review Committee 1985). In a 90-day study in rabbits, there was no indication that di-*n*-butyl phthalate was a skin sensitizer (Lehman 1955).

No studies regarding the following health effects in humans or experimental animals after dermal exposure to di-*n*-butyl phthalate were located:

3.2.3.4 Neurological Effects**3.2.3.5 Reproductive Effects****3.2.3.6 Developmental Effects****3.2.3.7 Cancer****3.2.4 Other Routes of Exposure**

No relevant studies for other routes of exposure were located.

3.3 GENOTOXICITY

Genotoxic Effects. Available *in vitro* genotoxicity data are summarized in Table 3-3. Di-*n*-butyl phthalate has tested negative or marginally positive in prokaryotic (Agarwal et al. 1985; Seed 1982) and eukaryotic (Barber et al. 2000) gene mutation, chromosomal aberration (Ishidate and Odashima 1977), and DNA damage (Kleinsasser et al. 2000) studies. These results suggest that di-*n*-butyl phthalate may be weakly mutagenic *in vitro*. The significance of these findings to the intact mammalian organism is not known because *in vivo* genotoxicity studies have not been conducted.

Table 3-3. Genotoxicity of Di-n-butyl Phthalate *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
<i>Salmonella typhimurium</i>	Gene mutation	–	–	Florin et al. 1980; Rubin et al. 1979; Zeiger et al. 1985
<i>S. typhimurium</i>		–	(+)	Seed 1982
<i>S. typhimurium</i>		–	+	Agarwal et al. 1985
Eukaryotic organisms:				
Fungi:				
<i>Saccharomyces cerevisiae</i>	Gene mutation	–	–	Shahin and Von Borstel 1977
Mammalian cells:				
Human oropharyngeal mucosa	Single-strand DNA breaks	No data	+	Kleinsasser et al. 2000
Human nasal mucosa	Single-strand DNA breaks	No data	+	Kleinsasser et al. 2000
Mouse lymphoma	Gene mutation	+	–	Barber et al. 2000
Mouse lymphoma	Gene mutation	+	–	Hazleton Biotechnologies 1986
Chinese hamster ovary cells	Chromosomal aberrations	No data	(+)	Ishidate and Odashima 1977
Balb 3T3	Cell transformation	No data	–	Barber et al. 2000
Balb 3T3	Cell transformation	No data	–	Litton Bionetics 1985a

– = negative result; (+) = marginally positive; + = positive result

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3.4 TOXICOKINETICS**3.4.1 Absorption****3.4.1.1 Inhalation Exposure**

No studies regarding absorption in humans after inhalation exposure to di-*n*-butyl phthalate were located. The relatively low concentration of di-*n*-butyl phthalate found in the lungs of rats exposed to 50 mg/m³ of di-*n*-butyl phthalate for up to 6 months was suggested to indicate rapid absorption (Kawano 1980b). However, no metabolites were measured in this study, and so the lack of accumulation could be due to lung metabolism rather than absorption.

3.4.1.2 Oral Exposure

No studies regarding absorption in humans after oral exposure to di-*n*-butyl phthalate were located.

Studies in laboratory animals indicate that di-*n*-butyl phthalate is rapidly and extensively absorbed by the oral route. Extensive absorption is indicated by the fact that, in rats, 63–97% of an orally administered dose was accounted for in the urine within 24 hours after dosing (Foster et al. 1982; Tanaka et al. 1978; Williams and Blanchfield 1975). Forty-eight hours after dosing, 85–100% of an oral dose of ¹⁴C-di-*n*-butyl phthalate was excreted in the urine (Tanaka et al. 1978; Williams and Blanchfield 1975). Similar results were obtained in hamsters, where 73% of an orally administered dose of ¹⁴C-di-*n*-butyl phthalate was excreted in the urine within 24 hours (Foster et al. 1982). *In vitro* studies indicate that a metabolite of di-*n*-butyl phthalate, mono-*n*-butyl phthalate, is probably the main form absorbed through the intestine (Lake et al. 1977; Takahashi and Tanaka 1989).

3.4.1.3 Dermal Exposure

No studies regarding absorption in humans after dermal exposure to di-*n*-butyl phthalate were located, although *in vitro* studies using human skin indicate that slow absorption by this route might occur (Scott et al. 1987).

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In rats, approximately 60% of a single dermal dose of 157 $\mu\text{mol/kg}$ was excreted in the urine during a 7-day period (the di-*n*-butyl phthalate remained on the skin for 7 days). The rate of di-*n*-butyl phthalate excretion (percentage of dose in 24 hours) remained constant over 7 days at 10–12% (Elsisi et al. 1989). The *in vitro* rate of dermal absorption in rats has been shown to be much greater than in humans (human skin absorption was 0.8% that of rat skin) (Scott et al. 1987).

3.4.2 Distribution

There are no data on the distribution of di-*n*-butyl phthalate in humans. Animal data suggest that following inhalation, oral, or dermal exposure, di-*n*-butyl phthalate is widely distributed throughout the body and does not accumulate in the body. There are no data on transplacental transfer or transfer via maternal milk. There is some evidence to suggest that di-*n*-butyl phthalate and its metabolites are rapidly cleared from the body (Foster et al. 1982; Tanaka et al. 1978; Williams and Blanchfield 1975). Thus, it is unlikely that di-*n*-butyl phthalate will be stored in maternal tissues and released during pregnancy or lactation.

3.4.2.1 Inhalation Exposure

No studies regarding distribution in humans after inhalation exposure to di-*n*-butyl phthalate were located.

In rats exposed to di-*n*-butyl phthalate by inhalation for 3 or 6 months, di-*n*-butyl phthalate was detected in all organs examined from rats exposed at 50 mg/m^3 (Kawano 1980b). After 6 months of exposure, the highest concentrations were found in brain, followed by lung, kidney, liver, and testicles (Kawano 1980b). Organ concentrations varied considerably between rats. At exposure to 0.5 mg/m^3 , di-*n*-butyl phthalate was consistently detected only in brains of exposed rats (Kawano 1980b).

3.4.2.2 Oral Exposure

No studies regarding distribution in humans after oral exposure to di-*n*-butyl phthalate were located.

Studies in rats on the distribution of ^{14}C -labeled di-*n*-butyl phthalate indicate that it is distributed throughout the body and that no significant retention occurs in any organ (Tanaka et al. 1978; Williams

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and Blanchfield 1975). Evaluation of tissues for ^{14}C at intervals 4–48 hours after dosing showed no accumulation. At all of the time points evaluated, no organ contained >0.7% of the administered dose (Williams and Blanchfield 1975). Even when rats were fed 0.1% di-*n*-butyl phthalate in the diet for up to 12 weeks, no accumulation in any organs was observed (Williams and Blanchfield 1975).

Following a single oral dose of 1,500 mg/kg [^{14}C]-di-*n*-butyl phthalate to pregnant rats on gestational day 14, the amount of radioactivity in the embryo peaked at 0.12% of the total administered dose at 6 hours postdosing, and thereafter rapidly declined to undetectable levels (Saillenfait et al. 1998).

3.4.2.3 Dermal Exposure

No studies regarding distribution in humans following dermal exposure to di-*n*-butyl phthalate were located.

A study in rats indicated that there was little or no accumulation of di-*n*-butyl phthalate in the body 7 days after a single dermal application of 43.6 mg/kg of ^{14}C -labeled di-*n*-butyl phthalate (Elsisi et al. 1989). Though approximately 65% of the dose had been absorbed and eliminated, only small amounts were found in tissues. Of the administered dose, 1.4% was in the skin, 1.1% was in muscle, and 0.41% was in adipose tissue. All other tissues combined contained <0.5% of the dose. About 33% of the dose remained at the site of application.

3.4.3 Metabolism

There is no direct evidence in humans or animals that the metabolism of di-*n*-butyl phthalate differs between adults and children. However, the activity of glucuronosyltransferase, a phase II enzyme involved in the biotransformation of mono-*n*-butyl phthalate to mono-*n*-butyl phthalate glucuronide, differs between adults and infants; adult activity is achieved at 6–18 months of age (Leeder and Kearns 1997).

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3.4.3.1 Inhalation Exposure.

No studies regarding metabolism in humans or animals following inhalation exposure to di-*n*-butyl phthalate were located.

3.4.3.2 Oral Exposure

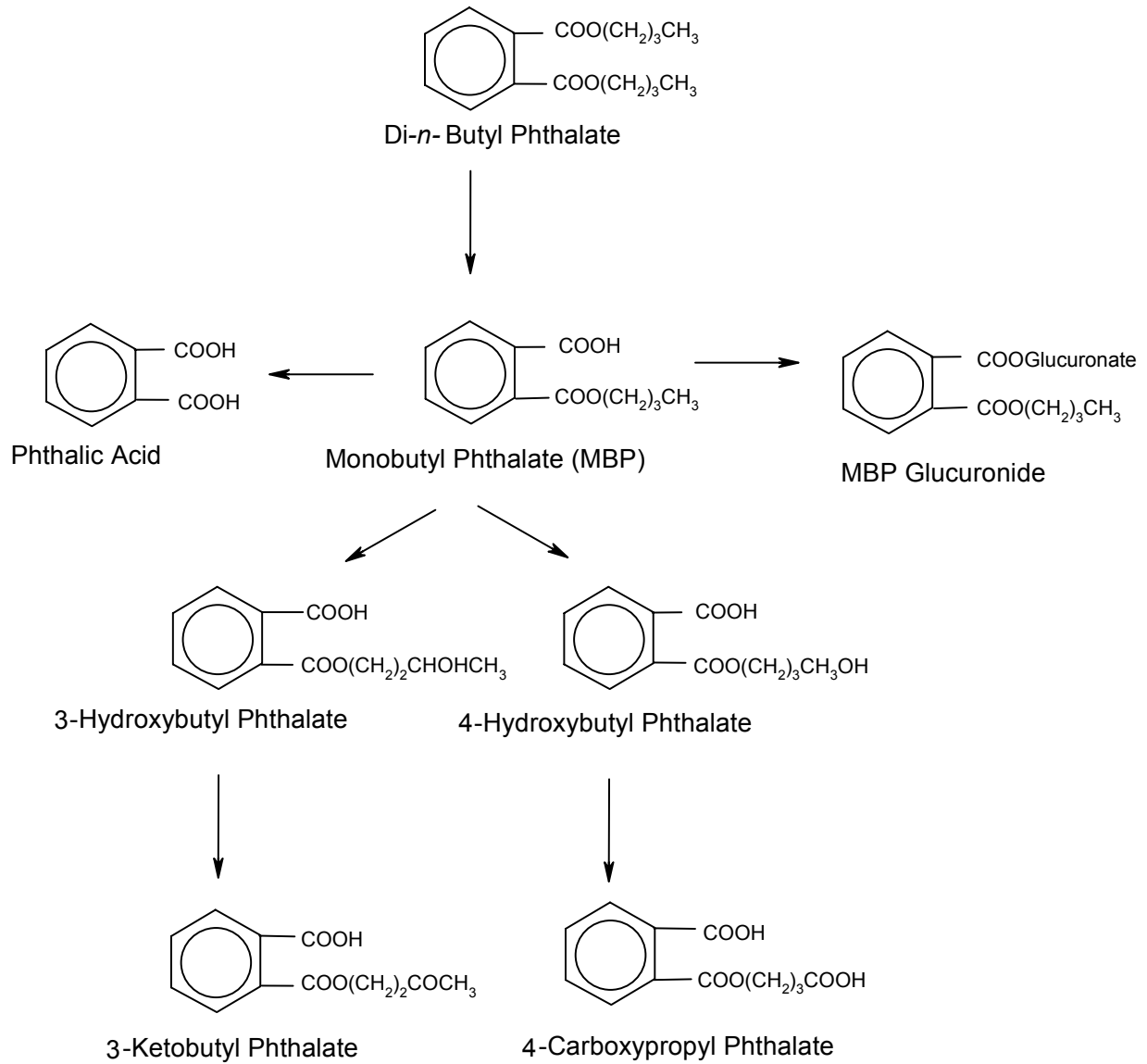
No studies regarding di-*n*-butyl phthalate metabolism in humans were located. Studies in animals indicate that metabolism of di-*n*-butyl phthalate proceeds mainly by hydrolysis of one butyl ester bond to yield mono-*n*-butyl phthalate. The product that appears in the urine is mainly mono-*n*-butyl phthalate conjugated with glucuronic acid, with lower levels of unconjugated mono-*n*-butyl phthalate, various oxidation products of mono-*n*-butyl phthalate (see Figure 3-2), and a small amount of the free phthalic acid (Figure 3-2) (Albro and Moore 1974; Foster et al. 1982; Kawano 1980b; Tanaka et al. 1978; Williams and Blanchfield 1975).

Species differences in the excretion of conjugated and unconjugated di-*n*-butyl phthalate in the urine of rats and hamsters have been identified by Foster et al. (1982). Rats excreted a larger proportion (14%) of the administered dose as unconjugated mono-*n*-butyl phthalate than hamsters, in which only 3.5% was excreted unconjugated. The authors indicated that this difference might explain why exposure to di-*n*-butyl phthalate causes greater testicular damage in rats than in hamsters (see Section 3.2.2.6 Developmental Effects).

3.4.3.3 Dermal Exposure

No studies regarding metabolism in humans and animals following dermal exposure to di-*n*-butyl phthalate were located.

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Figure 3-2. Metabolic Scheme for Di-*n*-butyl Phthalate in Animals

Source: Adapted from Albro and Moore 1974; Foster et al. 1982; Tanaka et al. 1978

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3.4.4 Elimination and Excretion**3.4.4.1 Inhalation Exposure**

No studies regarding excretion in humans or animals following inhalation exposure to di-*n*-butyl phthalate were located.

3.4.4.2 Oral Exposure

No studies regarding excretion in humans following oral exposure to di-*n*-butyl phthalate were located.

Studies in laboratory animals (rats, hamsters, and guinea pigs) indicate that 63–97% of an oral dose of di-*n*-butyl phthalate is eliminated in the urine within 24 hours, with 85–100% recovered by 48 hours (Foster et al. 1982; Tanaka et al. 1978; Williams and Blanchfield 1975). The fraction of the dose that was not accounted for in the urine was present in the feces. Excretion was essentially complete by 48 hours after administration of a single oral dose (Tanaka et al. 1978)

3.4.4.3 Dermal Exposure

No studies were located regarding excretion in humans following dermal exposure to di-*n*-butyl phthalate.

In rats, following a single dermal application of ¹⁴C-labeled di-*n*-butyl phthalate, 10–12% of the administered dose was excreted in urine and 1% was excreted in the feces (Elsisi et al. 1989). Seven days after application, 60% of the applied dose had been excreted.

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

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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).

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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 3-3 shows a conceptualized representation of a PBPK model.

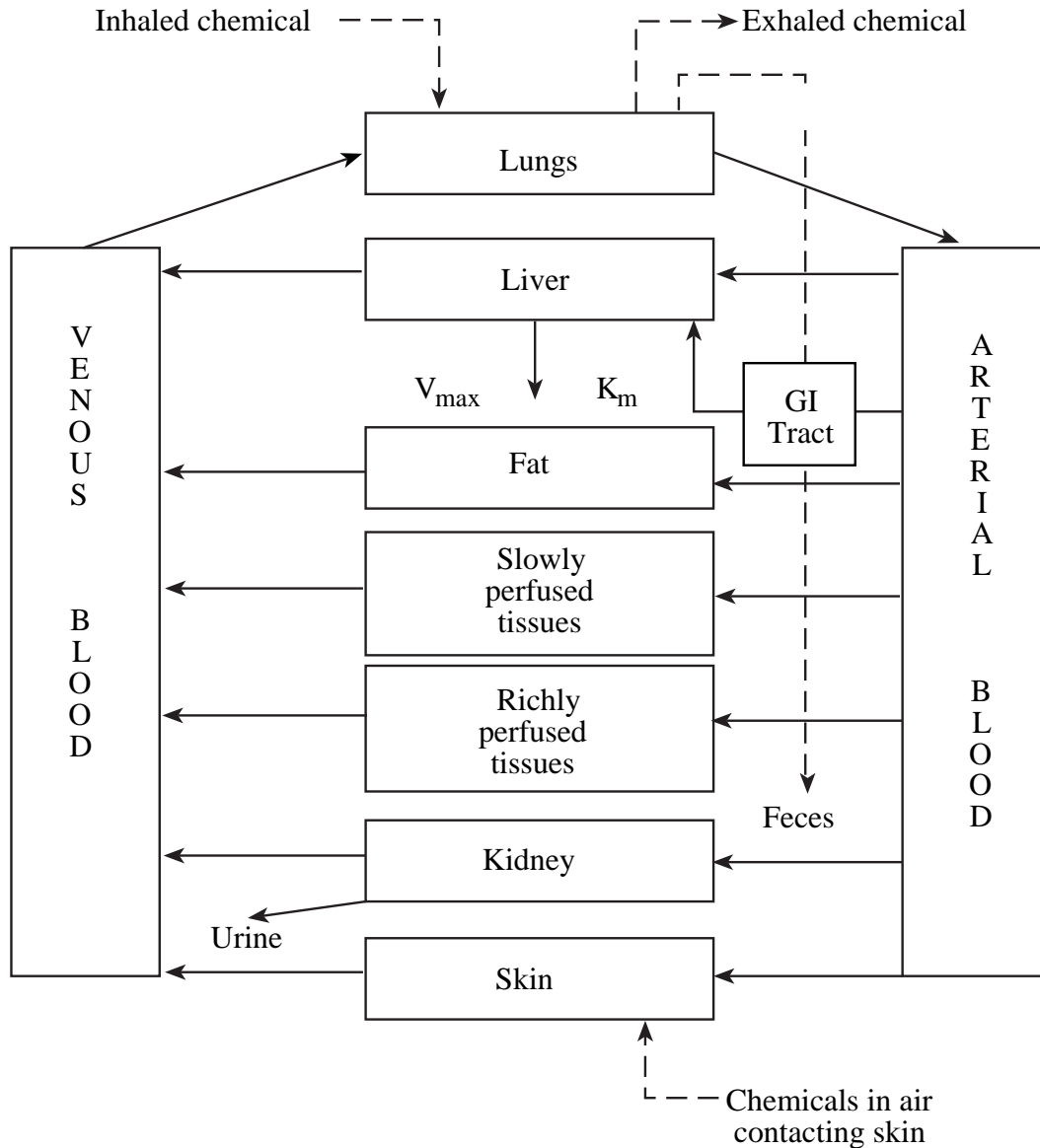
Keys et al. (2000) describe a PBPK model of di-*n*-butyl phthalate in rats that simulates the pharmacokinetics of both di-*n*-butyl phthalate and its major metabolite, mono-*n*-butyl phthalate. The model is intended for use in simulating doses of mono-*n*-butyl phthalate to the testes resulting from oral exposures to di-*n*-butyl phthalate. It is based on a earlier model developed for simulating the pharmacokinetics of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate (Keys et al. 1999).

Description of the Model. The Keys et al. (2000) model simulates seven tissue compartments: small intestine, blood, liver, fat, testis, slowly perfused tissues, and rapidly perfused tissues. The model simulates the absorption of mono-*n*-butyl phthalate formed from di-*n*-butyl phthalate in the small intestine; absorption of intact di-*n*-butyl phthalate is assumed not to occur. Conversion of di-*n*-butyl phthalate to mono-*n*-butyl phthalate in the small intestine is simulated with a first order rate constant. Absorption of mono-*n*-butyl phthalate from the small intestine is simulated with a first order rate constant for uptake into the liver. Elimination of absorbed mono-*n*-butyl phthalate is assumed to be entirely by metabolism in the liver. Metabolism of mono-*n*-butyl phthalate is simulated with a single Michaelis-Menten-type function (i.e., k_m and V_{max}), which represents all pathways combined.

Keys et al. (2000) explored five approaches to modeling the pharmacokinetics of di-*n*-butyl phthalate and mono-*n*-butyl phthalate. In a flow-limited version of the model, transfers between blood and tissues are simulated as functions of blood flow, tissue concentrations of di-*n*-butyl phthalate or mono-*n*-butyl phthalate, and tissue: blood partition coefficients, assuming instantaneous partitioning of the compounds between tissue and blood (Ramsey and Anderson 1984). In an *enterohepatic circulation* version of the model, the transfer of mono-*n*-butyl phthalate 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 mono-*n*-butyl phthalate from the small intestine. 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 requires 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

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Figure 3-3. 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.

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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. (2000). In a *pH-trapping* version of the model, instantaneous partitioning (i.e., flow-limited) of only the nonionized species of mono-*n*-butyl phthalate between the intracellular and extracellular compartments of tissues is assumed, and the respective concentrations of the nonionized and ionized species in each compartment are predicted by the pK_a for the carboxylic acid moiety of mono-*n*-butyl phthalate (pK_a is assumed to be 3.79, and intracellular pH is assumed to be approximately 7.0). In a *diffusion-limited, pH-trapping* version of the model, mono-*n*-butyl phthalate is assumed to ionize and reach equilibrium with its nonionized form, as in the pH trapping version; however, the rate of partitioning of the nonionized mono-*n*-butyl phthalate from the extracellular to the intracellular compartment of the tissue is controlled by the permeation coefficient-surface area-cross product (diffusion-limited). In the intracellular compartment, mono-*n*-butyl phthalate is assumed to equilibrate, where it was mostly ionized. The permeation coefficient-surface area-cross product combined with the nonionized mono-*n*-butyl phthalate tissue:blood partition coefficients control the rate at which nonionized mono-*n*-butyl phthalate is predicted to leave the intracellular compartment.

Tissue:blood partition coefficients for total and nonionized mono-*n*-butyl phthalate were estimated from their *n*-octanol:water partition coefficients (K_{ow}), using the approach reported by Poulin and Krishnan (1995). Tissue:blood partition coefficients for total mono-*n*-butyl phthalate (ionized and nonionized) were determined experimentally using a vial-equilibration method with correction for pH (Table 3-4).

Keys et al. (2000) 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 di-*n*-butyl phthalate and mono-*n*-butyl phthalate. The parameters estimated included the k_m and V_{max} values for metabolism of mono-*n*-butyl phthalate in the liver, the first order rate constant for the metabolism of di-*n*-butyl phthalate in the intestine, the first-order rate constant for absorption of mono-*n*-butyl phthalate from the small intestine, intracellular-to-extracellular transfer constants (e.g., permeation coefficient-surface-area-cross product) of nonionized mono-*n*-butyl phthalate, and biliary transfer of mono-*n*-butyl phthalate from liver to small intestine (these values are not provided in the profile because they are derived from optimization procedures and may not be directly useful for other models). Keys et al. (2000) do not explicitly cite or describe the data sets used to optimize model

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Table 3-4. Tissue:Blood Partition Coefficients Used in the Keys et al. (2000) Model

Tissue	Nonionized mono- <i>n</i> -butyl phthalate (estimated) ^a	Total mono- <i>n</i> -butyl phthalate (experimental) ^b	Total mono- <i>n</i> -butyl phthalate (estimated) ^c
Liver	15.8	1.22 ± 0.25	0.9
Fat	313.0	0.05 ± 0.5	0.9
Muscle	4.6	Negative	1.9
Testes	4.9	1.9 ± 0.21	4.9

^afrom K_{ow} based on algorithms from Poulin and Krishnan (1995); used for the pH trapping and combined diffusion limited-pH trapping models

^bvial equilibration study with pH correction

^cfrom K_{ow} at physiological pH (largely ionized) based on algorithms from Poulin and Krishnan (1995); used for the flow limited, enterohepatic circulation, and diffusion limited models

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parameter values. Based on Table 4 of their report, it appears that at least some data from National Institute of Environmental Health Sciences (NIEHS) (1994, 1995) were used to optimize the model.

Validation of the Model. Outputs from the various models were compared to observations of blood concentrations reported from studies of oral gavage or intravenous exposures of rats to di-*n*-butyl phthalate (NIEHS 1994, 1995). Based on the comparisons of model outputs to observed time courses for blood mono-*n*-butyl phthalate concentrations from NIEHS (1994, 1995), Keys et al. (2000) concluded that the *diffusion-limited, 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 4 of Keys et al. 2000). The *diffusion-limited, pH-trapping* model simulated reasonably well the time courses for blood concentrations of mono-*n*-butyl phthalate reported by NIEHS (1994, 1995). A log-likelihood ratio test was used to compare the fit of the various augmented models to that of the flow-limited model. The *diffusion-limited, pH-trapping* model gave a better statistical fit to the empirical data than the other four models, with the next best fit achieved with enterohepatic circulation model. However, the latter model appeared to underestimate peak mono-*n*-butyl phthalate plasma concentrations, which would be an important limitation for its use in risk assessment.

Risk Assessment. The model provides an approach to estimating doses of mono-*n*-butyl phthalate to the testes of the rat following oral doses of di-*n*-butyl phthalate and may 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 mono-*n*-butyl phthalate.

Species Extrapolation. The model is designed to predict the blood and testes concentrations of mono-*n*-butyl phthalate following oral doses of di-*n*-butyl phthalate 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.

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Interroute Extrapolation. The model is designed to simulate the pharmacokinetics of di-*n*-butyl phthalate and its metabolite, mono-*n*-butyl phthalate, when exposure is by the oral route. The pharmacokinetics of di-*n*-butyl phthalate 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 may 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

There is good evidence that following oral exposure, di-*n*-butyl phthalate is metabolized to mono-*n*-butyl phthalate and butanol by nonspecific esterases in the gastrointestinal tract (Cater et al. 1977; Lake et al. 1977; Takahashi and Tanaka 1989). The results of studies by Fukuoka et al. (1995) and Zhou et al. (1990) suggest that mono-*n*-butyl phthalate may be the chemical responsible for the testicular toxicity of di-*n*-butyl phthalate.

3.5.2 Mechanisms of Toxicity

The most characteristic effect of di-*n*-butyl phthalate in animal models is the effect on the testes, in particular the testicular atrophy observed following acute- (Cater et al. 1977; Fukuoka et al. 1989, 1990; Gray and Gangolli 1986; Oishi and Hiragi 1980b) or intermediate- (Gray et al. 1999; NTP 1995; Srivastava et al. 1990) duration exposure or *in utero*/lactational exposure (IRDC 1984; Mylchreest et al. 1998b, 2000; NTP 1995). The testicular atrophy is characterized by sloughing of germ cells as early as 6-hours post exposure (Fukuoka et al. 1990), ultimately resulting in seminiferous tubules with only Sertoli cells.

Fukuoka and associates have conducted a series of studies to elucidate the mechanism of testicular toxicity (Fukuoka et al. 1989, 1990, 1993, 1994, 1995; Zhou et al. 1990). One proposed mechanism of toxicity involves a disturbance in the interaction between germ cells and Sertoli cells. The Sertoli cell-germ cell interaction is generally considered to be required for the differentiation of male germ cells and their progression through the seminiferous epithelium and release as mature spermatozoa. Exposure to

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di-*n*-butyl phthalate is associated with both the release of iron from hemoglobin and/or transferrin in the liver and spleen, and the subsequent depletion of iron in the blood and testes. The decreased amount of available iron results in a decrease in succinate dehydrogenase activity in the Sertoli cells, resulting in disturbances in the energy transfer system between Sertoli cells and germ cells; anoxia due to iron depletion and/or disturbances in the energy supply may induce the sloughing of germ cells. Decreases in testicular sorbitol, fructose, and glucose levels have been observed in the testes 3–12 hours post exposure. Two days after exposure, there were significant decreases in sorbitol dehydrogenase and succinate dehydrogenase activities and decreases in testicular iron and zinc levels. Another mechanism of toxicity proposed for all phthalate esters that cause testicular toxicity involves interference with the interaction of follicle stimulating hormone (FSH) with the FSH receptor on Sertoli cells (NTP 2000). This mechanism may be applicable to di-*n*-butyl phthalate, but no studies regarding this mechanism and di-*n*-butyl phthalate were located. It is likely that mono-*n*-butyl phthalate, the primary metabolite of di-*n*-butyl phthalate, is responsible for the testicular toxicity.

3.5.3 Animal-to-Human Extrapolations

Species differences in the testicular toxicity of di-*n*-butyl phthalate have been observed. Severe testicular atrophy has been observed in rats and guinea pigs orally exposed to di-*n*-butyl phthalate. In mice exposed to the same oral dose, focal testicular atrophy was observed, and no testicular effects were observed in hamsters (Gray et al. 1982). The basis for the species differences is not known, but could be related to species differences in the free concentration of the primary metabolite of di-*n*-butyl phthalate, mono-*n*-butyl phthalate (Foster et al. 1982). In rats, the levels of mono-*n*-butyl phthalate and di-*n*-butyl phthalate and β -glucuronidase activity in the testes were markedly higher than in hamsters. Foster et al. (1982) suggested that the rate of mono-*n*-butyl phthalate glucuronide hydrolysis in rats is higher than in hamsters resulting in increased mono-*n*-butyl phthalate levels in the rat testes. There are insufficient data to assess whether rats, mice, or hamsters would be an appropriate animal model for testicular toxicity in humans.

Studies in nonhuman primates with phthalate esters (primarily di-isononyl phthalate and di-2-ethylhexyl phthalate) have shown no hepatic effects similar to those seen in rodents, including peroxisome proliferation (Astill 1989; Pugh et al. 2000; Rhodes et al. 1986; Short et al. 1987); this suggests that primates, including humans, are probably not sensitive to the hepatic effects of peroxisome proliferators.

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3.6 ENDOCRINE DISRUPTION

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, or otherwise interfere with the normal function of the endocrine system. Chemicals with this type of activity are most commonly referred to as endocrine disruptors. Some scientists believe that chemicals with the ability to disrupt the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. Others believe that endocrine disrupting chemicals do not pose a significant health risk, particularly in light of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavonoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These compounds are derived from plants and are similar in structure and action as endogenous estrogen. While there is some controversy over the public health significance of endocrine disrupting chemicals, it is agreed that the potential exists for these compounds to affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior (EPA 1997). As a result, endocrine disruptors may play a role in the disruption of sexual function, immune suppression, and neurobehavioral function. Endocrine disruption is also thought to be involved in the induction of breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Phthalates are a class of chemicals that have been implicated as having estrogenic properties. There is both *in vitro* and *in vivo* evidence of the weak estrogenic behavior of di-*n*-butyl phthalate. The results of three *in vitro* assays provide evidence of weak estrogenic activity. The first study used the recombinant yeast screening test to assess estrogenic activity (Harris et al. 1997). In this test, a gene for human estrogen receptor is integrated into the main yeast genome and is expressed in a form capable of binding to estrogen response elements and controlling the expression of the reporter gene *lac-Z*; when the *lac-Z* gene is expressed, β -galactoside is produced. Harris et al. (1997) found a dose-dependent increase in β -galactosidase production suggesting that di-*n*-butyl phthalate had some estrogenic activity. However, the di-*n*-butyl phthalate was approximately 10-million-fold less potent than estradiol. Di-*n*-butyl phthalate also induced proliferation of MCF-7 and ZR-75 human breast cancer cell lines (Harris et al. 1997). Zacharewski et al. (1998) found that di-*n*-butyl phthalate induced reporter gene (luciferase) activity in MCF-7 human breast cancer cells, but not HeLa human cervical carcinoma cells, transfected with human estrogen receptor, although approximately 3,000-fold less potent than 17β -estradiol. Additionally, Zacharewski et al. (1998) found that di-*n*-butyl phthalate was capable of supporting a very

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modest amount of growth in an estrogen-dependent recombinant yeast strain. Jobling et al. (1995) found that di-*n*-butyl phthalate reduced the binding of 17 β -estradiol to the fish estrogen receptor and stimulated the transcriptional activity of the estrogen receptor. Zacharewski et al. (1998) found that di-*n*-butyl phthalate was also capable of competing weakly with 17 β -estradiol for binding to Sprague-Dawley rat uterine estrogen receptor *in vitro*, although over 36,000 times weaker than 17 β -estradiol. However, differences in the kinetics of displacement of estrogen from the fish and rat estrogen receptors by di-*n*-butyl phthalate may indicate significant species differences between estrogen receptors for binding di-*n*-butyl phthalate (Jobling et al. 1995; Zacharewski et al. 1998). Although the results of the *in vitro* studies consistently suggest that di-*n*-butyl phthalate has estrogenic properties, the *in vivo* data do not provide supportive evidence for di-*n*-butyl phthalate estrogenicity. This may be due, at least in part, to the presence *in vivo* of esterases that metabolize di-*n*-butyl phthalate to mono-*n*-butyl phthalate, which has been reported not to interact with the estrogen receptor (Mylchreest et al. 1998b). An acute *in vivo* assay by Milligan et al. (1998) did not find a significant increase in uterine vascular permeability in ovariectomized mice 4 hours after subcutaneous administration of 927 mg/kg di-*n*-butyl phthalate. In contrast to 17 β -estradiol, di-*n*-butyl phthalate did not affect body weight, induce an increase in uterine wet weight, or induce vaginal cornification in ovariectomized rats treated orally with up to 2,000 mg/kg/day di-*n*-butyl phthalate for 4 days (Zacharewski et al. 1998). Likewise, di-*n*-butyl phthalate did not induce lordosis behavior or increased uterine weight in ovariectomized rats after exposure for 2 days, followed by 0.5 mg progesterone (Gray et al. 1999). Ema et al. (2000a) found that di-*n*-butyl phthalate failed to induce a decidual cell response in rats. Pseudopregnant rats (females bred to vasectomized males) treated di-*n*-butyl phthalate on days 0–8 of pseudopregnancy, followed by surgical induction of the decidual cell response had lower ovarian weights, uterine weights, and serum progesterone levels on day 9 of pseudopregnancy, compared to controls, indicating a lack of estrogenic activity. However, the results of the NTP (1995) multigeneration study, particularly the finding that the reproductive effects in the first generation males appeared more adverse than those in the parental generation (see Sections 2.2.2.5 Reproductive Effects and 3.2.2.6 Developmental Effects for a description of these effects), are suggestive that di-*n*-butyl phthalate can disrupt normal male development. Additionally, the decreases in anogenital distance observed in rat fetuses (Ema et al. 1998, 2000b; Mylchreest et al. 1999, 2000) suggest that di-*n*-butyl phthalate has anti-androgenic properties. This is further supported by the findings of Gray et al. (1999), showing similar, but not identical, effects from di-*n*-butyl phthalate exposure as from exposure to linuron, a known androgen receptor ligand. These effects included delayed preputial separation, reduced fertility, testicular atrophy, and reduced sperm production in treated males, and reduced anogenital distance, increased number of retained nipples, and

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decreased androgen-dependent tissue weights in male offspring (exposed *in utero* and via lactation only) of treated rats. However, these androgen-related effects do not appear to be mediated by interaction of di-*n*-butyl phthalate or its primary metabolite, mono-*n*-butyl phthalate, with the androgen receptor (Mylchreest et al. 1998b, 1999) (see Section 3.5.2 Mechanisms of Toxicity for a discussion of the possible mechanism of androgen disruption). Di-*n*-butyl phthalate is known to be a testicular toxicant. One mechanism of toxicity proposed for all phthalate esters that cause testicular toxicity involves interference with the interaction of follicle stimulating hormone (FSH) with the FSH receptor on Sertoli cells (NTP 2000). This mechanism may be applicable to di-*n*-butyl phthalate, but no studies regarding this mechanism and di-*n*-butyl phthalate were located.

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 are not small adults. They 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 5.6 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,

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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 while 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).

No information on the toxicity of di-*n*-butyl phthalate in children was located, and there is a limited amount of data in adults. The available oral-exposure animal studies clearly demonstrate that the developing organism is sensitive to the toxicity of di-*n*-butyl phthalate. Postimplantation losses and decreases in the number of live fetuses have been observed in a number of acute-duration studies in which rats received gavage doses of 630 mg/kg/day and higher on gestational days 0–8, 7–15, 7–9, 10–12, 13–15, or 6, 8, 10, 12, 13, 14, 15, or 16 (Ema et al. 1993, 1994, 1995, 1997a, 2000a) or in mice receiving gavage doses of 2,500 mg/kg/day on gestational days 6–13 (Hardin et al. 1987). Decreases in the number of live pups per litter have also been observed in intermediate-duration studies in which rats or mice were exposed to 80 or 1,950 mg/kg/day, respectively, throughout gestation, lactation, and postweaning (NTP 1995), or rats were exposed to 250–500 mg/kg/day *in utero* and during lactation (Gray et al. 1999). Decreases in fetal/pup body weight have been observed in rats receiving gavage doses of 500 mg/kg/day

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or higher di-*n*-butyl phthalate on gestational days 0–8 (Ema et al. 2000a), 630 or 750 mg/kg/day on gestational days 7–15, 7–9, 10–12, 13–15, 6, 7, 8, 9, 10, 11, or 16 (Ema et al. 1994, 1995, 1997a), and in rats and mice exposed to 240 or 460 mg/kg/day, respectively, in the diet throughout gestation, lactation, and postnatal days 28–56 (NTP 1995). Skeletal (primarily fusion of cervical vertebrae) and external (cleft palate) malformations have been observed in rats receiving gavage doses of 750 mg/kg/day on gestational days 7–9 or 13–15 (Ema et al. 1994, 1995). Impaired development of the reproductive system has also been observed in an acute-duration study (Ema et al. 1998) and in several intermediate-duration studies (IRDC 1984; Mylchreest et al. 1998b; NTP 1995). Ema et al. (1998) reported an increased incidence of undescended testes and decreased anogenital distance in the offspring of rats receiving gavage doses of 555 mg/kg/day and higher on gestational days 11–21. The reproductive effects observed in the intermediate-duration studies are similar to the effects observed in adult animals exposed to di-*n*-butyl phthalate. Decreases in reproductive organ weights, testicular degeneration/ atrophy, and hypospermia have been observed in the offspring of rats exposed throughout gestation, lactation, and postnatally to 250 mg/kg/day and higher di-*n*-butyl phthalate (Gray et al. 1999; IRDC 1984; Mylchreest et al. 1998b, 2000; NTP 1995). More details about these studies can be found in Section 3.2.2.6 Developmental Effects.

NTP (1995) conducted two studies to assess the impact of perinatal exposure on the subchronic toxicity of di-*n*-butyl phthalate. In the first study, weanling rats and mice were exposed to dietary di-*n*-butyl phthalate for 13 weeks. In the second study, the 13-week dietary exposure was immediately preceded by gestational and lactational exposure. Similar effects, particularly decreases in body weight gain, hepatomegaly, and testicular degeneration/atrophy were observed in both studies; suggesting that the perinatal rat is neither resistant nor hypersensitive to the short-term toxic effects of di-*n*-butyl phthalate as compared to rats only exposed postnatally (NTP 1995).

No human or animal data were located that examined possible age-related differences in the toxicokinetics of di-*n*-butyl phthalate. However, it is known that one of the enzymes involved in phase II biotransformation of mono-*n*-butyl phthalate, the primary di-*n*-butyl phthalate metabolite, is influenced by age. Glucuronosyltransferase activity differs in adults and children under the age of 6–18 months (Leeder and Kearns 1997). Di-*n*-butyl phthalate and its primary metabolite, mono-*n*-butyl phthalate, have been detected in placental and embryonic tissues of treated pregnant rats (Saillenfait et al. 1998), with mono-*n*-butyl phthalate accounting for 50–95% of the total amount recovered. In the embryo, approximately 1% of the recovered mono-*n*-butyl phthalate was conjugated with glucuronic acid, but

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approximately 10% was conjugated in maternal plasma and placenta; this may indicate limited transfer of conjugated mono-*n*-butyl phthalate to the embryo or an inability of embryonic tissues to conjugate mono-*n*-butyl phthalate. There are no data on the toxicokinetic properties of di-*n*-butyl phthalate in children or immature animals, or on transfer via maternal milk. Toxicokinetic information on di-*n*-butyl phthalate is sparse, but existing oral studies (Foster et al. 1982; Tanaka et al. 1978; Williams and Blanchfield 1975) indicate that di-*n*-butyl phthalate and its metabolites are rapidly cleared from the body. Thus, di-*n*-butyl phthalate from maternal preconception exposure is highly unlikely to be stored in maternal tissues and released during pregnancy or lactation.

Subsequent sections of this chapter (Sections 3.8, 3.9, and 3.11) discuss the available information on biomarkers, interactions, and methods for reducing toxic effects. The available information is from adults and mature animals; no child-specific information was identified. It is likely that this information will also be applicable to 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

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copper, zinc, and selenium). Biomarkers of exposure to di-*n*-butyl phthalate 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., 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 di-*n*-butyl phthalate 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 Di-*n*-butyl Phthalate

The presence of di-*n*-butyl phthalate has been reported in a number of human tissues and fluids. Di-*n*-butyl phthalate has been found in adipose tissue obtained from surgical procedures or autopsies (Mes et al. 1974; EPA 1986g), in lipid-rich atherosclerotic plaques (Ferrario et al. 1985), in seminal fluid (Murature et al. 1987), and in blood serum (Ching et al. 1981a; EPA 1986g). No study identified the source, amount, or duration of exposure to di-*n*-butyl phthalate associated with levels in the body. A study comparing surgical patients having known plasticizer exposure from intravenous bags and tubing with controls without known exposure found no correlation between exposure and serum levels of di-*n*-butyl phthalate (Ching et al. 1981a). There was no quantitative relationship between the concentration of di-*n*-butyl phthalate in seminal fluid and sperm count (Murature et al. 1987). Thus, measurements of di-*n*-butyl phthalate in body tissues and fluids can indicate that exposure has taken place, but not the amount or duration of exposure. No data were found on the concentration of the primary metabolite, mono-*n*-butyl phthalate, in human body tissues or fluids.

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3.8.2 Biomarkers Used to Characterize Effects Caused by Di-*n*-butyl Phthalate

Effects caused by di-*n*-butyl phthalate exposure in animals include liver changes and effects on development and reproduction. None of these effects appear to be specific to di-*n*-butyl phthalate exposure. Liver changes, such as altered enzymatic activity and peroxisome proliferation, are induced by many other chemicals (Moody et al. 1991; Popp et al. 1989). These and other effects associated with di-*n*-butyl phthalate exposure do not appear to be sufficiently specific to serve as biomarkers of effects.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Administration of zinc provides some protection against the testicular toxicity of di-*n*-butyl phthalate exposure in rats (Cater et al. 1977). No other studies were located regarding the interaction of di-*n*-butyl phthalate with other chemicals. Schulsinger and Mullgaard (1980) reported that humans exposed to a mixture of three phthalate esters, including di-*n*-butyl phthalate, did not develop dermal sensitization, but since di-*n*-butyl phthalate is negative for skin sensitization, these results shed little light on possible interactions.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to di-*n*-butyl phthalate than will most persons exposed to the same level of di-*n*-butyl phthalate 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 result in reduced detoxification or excretion of di-*n*-butyl phthalate, or compromised function of organs affected by di-*n*-butyl phthalate. Populations who are at greater risk due to their unusually high exposure to di-*n*-butyl phthalate are discussed in Section 6.7 Populations with Potentially High Exposures.

There are no data in humans to suggest that any segment of the human population is unusually susceptible to the effects of di-*n*-butyl phthalate. However, in animal studies, a number of developmental effects were reported, including postimplantation losses, decreases in the number of live fetuses, decreases in fetal/pup body weight, increases in the incidence of external and skeletal malformations, and impaired development of the reproductive system (Ema et al. 1993, 1994, 1995a, 1997a, 1998, 2000a, 2000b; Gray et al. 1999; IRDC 1984; Mylchreest et al. 1998b, 1999, 2000; NTP 1995). In most of these studies, the developmental

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effects were at dietary or gavage levels which were not associated with maternal toxicity. This suggests that the fetus may be somewhat more susceptible to di-*n*-butyl phthalate than the adult, and that it may be prudent to consider pregnant females more susceptible to di-*n*-butyl phthalate than other adults. Some studies suggested that pregnant rats may be more susceptible to the lethal effects of di-*n*-butyl phthalate. A more detailed discussion of children's susceptibility can be found in Section 3.7.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to di-*n*-butyl phthalate. 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 di-*n*-butyl phthalate. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

No texts were found that provided specific information about treatment following exposures to di-*n*-butyl phthalate

3.11.1 Reducing Peak Absorption Following Exposure

Methods for reducing peak absorption of di-*n*-butyl phthalate include gut dilution, eye irrigation, and washing with soap (Ellenhorn 1997). No information regarding ways to reduce absorption following inhalation exposure was located.

3.11.2 Reducing Body Burden

No experimental data regarding methods for reducing the di-*n*-butyl phthalate body burden were located. The available toxicokinetic data suggest that di-*n*-butyl phthalate is rapidly cleared from the body. Twenty-four hours after dosing, 63–97% of an oral dose is excreted in the urine (Foster et al. 1982; Tanaka et al. 1978; Williams and Blanchfield 1975).

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3.11.3 Interfering with the Mechanism of Action for Toxic Effects

No methods which would interfere with mechanism of di-*n*-butyl phthalate toxic were identified.

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 di-*n*-butyl phthalate 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 di-*n*-butyl phthalate.

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 Di-*n*-butyl Phthalate

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to di-*n*-butyl phthalate are summarized in Figure 3-4. The purpose of this figure is to illustrate the existing information concerning the health effects of di-*n*-butyl phthalate. 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 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* (ATSDR 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.

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Figure 3-4. Existing Information on Health Effects of Di-*n*-butyl Phthalate

		Systemic									
		Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
Inhalation				•		•					
Oral											
Dermal					•						

Human

		Systemic									
		Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
Inhalation		•	•		•	•	•				
Oral		•	•	•		•	•	•		•	
Dermal		•	•	•	•						

Animal

- Existing Studies

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Limited data are available on effects in humans, consisting of an occupational study of workers exposed to mixtures of plasticizers and dermal sensitization studies conducted to evaluate the effects of di-*n*-butyl phthalate in cosmetic products. Data from animal studies are more extensive. As a result of early findings on testicular effects of di-*n*-butyl phthalate, most studies have tended to concentrate mainly on developmental and reproductive effects. A few studies provide data on systemic effects, but since these appear to be minor, research in this area has not been extensive. No data are available on the chronic effects of di-*n*-butyl phthalate, or on its carcinogenic potential.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. No information regarding health effects of di-*n*-butyl phthalate in humans was located. Animal studies that have examined the acute toxicity of orally administered di-*n*-butyl phthalate have primarily focused on reproductive (Cater et al. 1977; Fukuoka et al. 1989, 1990; Gray et al. 1982; Oishi and Hiraga 1980b; Tanino et al. 1987) and developmental (Ema et al. 1993, 1994, 1995a, 1997b, 1998; Saillenfait et al. 1998) end points. An acute-duration oral MRL was derived from a NOAEL for developmental effects (Mylchreest et al. 2000). The systemic toxicity of di-*n*-butyl phthalate has not been adequately assessed. No information concerning target organs following acute-duration inhalation or dermal exposure to di-*n*-butyl phthalate in animals or humans was located, and no acute inhalation MRL could be derived. Additional information concerning the target organs and mechanism of toxicity of di-*n*-butyl phthalate exposure by the inhalation, oral, and dermal routes are needed to assess the risks to populations surrounding hazardous waste sites that might be exposed to di-*n*-butyl phthalate for brief periods.

Intermediate-Duration. No human data on the toxicity of di-*n*-butyl phthalate following intermediate-duration exposure were identified. Systemic, reproductive, and developmental effects have been observed in animals following oral exposure. The liver appears to be the most sensitive systemic target in rats and mice exposed to di-*n*-butyl phthalate in the diet for 13 weeks (NTP 1995; Schilling et al. 1992). The reproductive effects consist of testicular atrophy with decreases in spermatogenesis (Murakami et al. 1986a, 1986b; NTP 1995; Srivastava et al. 1990), and the developmental effects included decreases in the number of pups per litter in rats and impaired reproductive development in male offspring (NTP 1995). An intermediate-duration oral MRL was not derived because the lowest LOAEL was identified for a serious effect and the study did not identify a NOAEL. A toxicity study

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identifying a NOAEL for developmental effects are needed for assessing risk for populations living near hazardous waste sites.

No intermediate-duration inhalation studies were identified. These data are needed for assessing risk associated with exposure to inhaled di-*n*-butyl phthalate. The only intermediate-duration dermal study (Lehman 1955) examined a very limited number of end points and would have limited usefulness for assessing hazard and risk following dermal exposure to di-*n*-butyl phthalate.

Chronic-Duration Exposure and Cancer. No information concerning the toxic effects of chronic-duration exposure to di-*n*-butyl phthalate in humans or animals by any route of exposure was located. Studies to establish the target organs and levels causing effects following chronic-duration exposure to di-*n*-butyl phthalate by inhalation, oral, and dermal exposure are needed to assess the risks to populations surrounding hazardous waste sites that might be exposed to di-*n*-butyl phthalate for long periods of time.

No information regarding the carcinogenicity of di-*n*-butyl phthalate in humans or animals was located.

Genotoxicity. A limited number of *in vitro* tests for genotoxicity suggest that di-*n*-butyl phthalate may have weak genotoxic potential. No *in vivo* studies have been conducted. *In vivo* genotoxicity studies are needed to determine whether di-*n*-butyl phthalate has mutagenic potential and, if so, what the possible mechanism of genotoxicity might be.

Reproductive Toxicity. No data on the reproductive toxicity of di-*n*-butyl phthalate in humans were located. Testicular atrophy and decreased fertility have been observed in orally-exposed animals (Cater et al. 1977; Fukuoka et al. 1989, 1990; Gray et al. 1982; Oishi and Hiraga 1980b; Tanino et al. 1987). Species differences are apparent, with rats and guinea pigs being more sensitive than mice, and hamsters being relatively insensitive to this effect (Gray et al. 1982). The species differences may be related to an increased ability to hydrolyze mono-*n*-butyl phthalate glucuronide resulting in increased levels of free mono-*n*-butyl phthalate and testicular toxicity. Information that could be used to determine which animal species would be a good model for testicular toxicity in humans would be useful in assessing risk in humans exposed to di-*n*-butyl phthalate. Further, studies administering chronic low levels of di-*n*-butyl phthalate are needed to determine whether or not any reproductive impairment or endocrine

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disruption occurs at background levels. Additionally, it is not known if testicular effects would also be a sensitive end point following inhalation or dermal exposure.

Developmental Toxicity. No human developmental toxicity studies were located. Studies in rats and mice have shown that oral exposure to di-*n*-butyl phthalate can result in a number of developmental effects including postimplantation loss, decreases in the number of live births/litter, external, internal, and skeletal malformations, and impaired development of the reproductive system in male offspring (Ema et al. 1993, 1994, 1995a, 1997b, 1998, 2000a, 2000b; Gray et al. 1999; IRDC 1984; Mylchreest et al. 1998b, 1999, 2000; NTP 1995; Saillenfait et al. 1998). Although a number of studies have examined the developmental toxicity of di-*n*-butyl phthalate, NOAELs have not been identified for some of the more sensitive developmental effects, such as decreases in the number of live births and impaired development of the reproductive system, following intermediate-duration oral exposure. Identification of NOAELs for these effects are needed to establish an intermediate-duration oral MRL for di-*n*-butyl phthalate. NTP (1995) conducted two studies to assess whether perinatal exposure increased the toxicity of di-*n*-butyl phthalate. The studies did not find any difference in terms of critical effects and doses; however, reproductive performance was not evaluated, and it is also not known if there would be any second generation developmental effects. Developmental end points have not been assessed in animals following inhalation or dermal exposure. Such studies are needed for extrapolating the possible risk to human populations exposed environmentally by these routes.

Immunotoxicity. The results from the available human and animal studies indicate that di-*n*-butyl phthalate is not a skin-sensitizing agent following dermal exposure (Lehman 1955; Schulsinger and Mollgaard 1980). These studies did not assess other aspects of immunotoxicity. Additionally, immunotoxicity has not been adequately assessed following inhalation or oral exposure. Tests of several additional end points of humoral and cell-mediated immune function are needed to assess the sensitivity of this system to di-*n*-butyl phthalate.

Neurotoxicity. The neurotoxic potential of di-*n*-butyl phthalate has not been adequately evaluated in human or animal studies. Neurotoxicity studies are needed to determine the hazards associated with human exposure to di-*n*-butyl phthalate.

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Epidemiological and Human Dosimetry Studies. Very limited epidemiological studies have been performed and generally involved exposure to a mixture of plasticizers at poorly-characterized levels; blood and tissue levels were the primary end points examined (Ferrario et al. 1985; Mes et al. 1974; Schulsinger and Mollgaard 1980).

Studies of people occupationally exposed to di-*n*-butyl phthalate are needed to assess the effects of di-*n*-butyl phthalate on human health. Since one of the most significant effects in animals is testicular atrophy, epidemiology studies of reproductive parameters in humans exposed to di-*n*-butyl phthalate would be particularly relevant. Such studies would be most valuable if dosimetry methods could be developed to provide reliable exposure data to accompany health effects data. This would assist in establishing cause/effect relationships and developing methods to monitor individuals living near hazardous waste sites.

Biomarkers of Exposure and Effect.

Exposure. The presence and concentration of di-*n*-butyl phthalate can be measured in a variety of biological tissues and fluids, but no information that would allow correlation of body levels with source, route, amount, or duration of exposure to di-*n*-butyl phthalate was located. The primary metabolite of di-*n*-butyl phthalate in several species is mono-*n*-butyl phthalate, and so mono-*n*-butyl phthalate or its glucuronide conjugate could possibly serve as a biomarker of exposure to di-*n*-butyl phthalate. However, because mono-*n*-butyl phthalate is also a metabolite of butyl benzyl phthalate (Nativelle et al. 1999), it is not a specific biomarker for di-*n*-butyl phthalate. Therefore, studies that identify specific markers of exposure and determine the relationship between body levels of di-*n*-butyl phthalate and exposure are needed to develop methods for identifying and monitoring populations with high exposure to di-*n*-butyl phthalate.

Effect. No known biomarkers of effect of di-*n*-butyl phthalate were identified. Studies to identify some early indication of impending injury to the male and female reproductive systems, perhaps based on the interference of zinc metabolism, would be valuable in assessing likely health consequences in people with above-average exposure to di-*n*-butyl phthalate.

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Absorption, Distribution, Metabolism, and Excretion. Studies in laboratory animals indicate that di-*n*-butyl phthalate given orally is readily absorbed, mainly as the metabolite mono-*n*-butyl phthalate, and subsequently, is rapidly excreted. Limited data exist regarding inhalation and dermal absorption. Studies on the absorption and metabolism of di-*n*-butyl phthalate by the inhalation and dermal routes are needed to evaluate human health risk by these routes of exposure.

Comparative Toxicokinetics. Syrian hamsters appear to be relatively resistant to the testicular effects of di-*n*-butyl phthalate compared to the rat. A comparative metabolic study with rats and hamsters indicated some quantitative differences between the two species with respect to the excretion of metabolites in the urine. Additional comparative studies, perhaps with other species, may add to our understanding of the mechanisms of toxicity to the male reproductive organs. Since it is well known that there are a wide variety of esterases with varying affinity for different substrates, further information on the substrate specificities of the esterases in various species, and on the enzymes involved in detoxification of di-*n*-butyl phthalate, especially glucuronosyltransferase, could help to understand the biological mechanisms behind the species differences in response to di-*n*-butyl phthalate.

Methods of Reducing Toxic Effects. Di-*n*-butyl phthalate is metabolized in the gastrointestinal tract by nonspecific esterases to form mono-*n*-butyl phthalate. Identification of substances that could inhibit this biotransformation and possibly reduce absorption or that would induce glucuronidation of mono-*n*-butyl phthalate would be valuable. There are no methods to block the toxic response due to exposure to di-*n*-butyl phthalate or to mitigate the observed health effects.

Children's Susceptibility. No information on the toxicity of di-*n*-butyl phthalate in children has been located. Studies that examine sensitive end points such as reproductive, hematological, or hepatic effects in young animals would be useful for assessing whether children will be unusually susceptible to di-*n*-butyl phthalate toxicity. It is particularly important to conduct studies on chronic low level exposure of di-*n*-butyl phthalate because of its ubiquitous nature in many everyday items. Further, research suggests di-*n*-butyl phthalate may have the capability to disrupt the endocrine system; such potential is especially critical in developing and prepubescent children. The available animal data suggest that the developing organism is sensitive to di-*n*-butyl phthalate toxicity. As discussed in Section 3.2.2.6, the observed developmental effects include postimplantation losses, decreases in the number of live fetuses per litter, decreases in fetal/pup body weights, increases in the incidences of external and skeletal malformations, and altered reproductive development in the offspring. Data needs relating to development are discussed

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in detail in the Developmental Toxicity subsection above. There are no data on whether di-*n*-butyl phthalate can cross the placenta or be transferred to an infant via breast milk. Although there are no data on whether di-*n*-butyl phthalate is stored in maternal tissues and whether these stores can be mobilized during pregnancy or lactation, the rapid clearance of di-*n*-butyl phthalate in animals receiving a single oral dose suggests that di-*n*-butyl phthalate would not be stored in maternal tissues.

The available toxicokinetic data did not evaluate the potential differences between adults and children, although there is some evidence that there are age-related differences in the activity of at least one enzyme, UDP-glucuronosyltransferase, that is involved in the metabolism of di-*n*-butyl phthalate. Toxicokinetic studies examining how aging can influence the absorption, distribution, and excretion of di-*n*-butyl phthalate would be useful in assessing children's susceptibility to di-*n*-butyl phthalate toxicity. The mechanism of action for a number of toxic effects have not been elucidated. There are no data to determine whether there are age-specific biomarkers of exposure or effects or any interactions with other chemicals that would be specific for children. There is very little available information on methods for reducing di-*n*-butyl phthalate toxic effects or body burdens; it is likely that research in adults would 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

Ongoing studies pertaining to di-*n*-butyl phthalate have been identified and are shown in Table 3-5.

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Table 3-5. Ongoing Studies on Di-*n*-butyl Phthalate

Investigator	Affiliation	Research description	Sponsor
Cunningham, ML	NIEHS, NIH	Evaluation of peroxisome proliferation-interspecies differences, induction of carcinogenicity	NIEHS
Pereira, MM	Medical College of Ohio	Effect of peroxisome proliferators on methylation of genes	NIEHS
Thomas, R	Florida A&M University	Genotoxicity of di- <i>n</i> -butyl phthalate	ATSDR

ATSDR = Agency for Toxic Substances and Disease Registry; NIEHS = National Institute of Environmental Health