2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of di-*n*-octylphthalate. 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.

2.2 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 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 or exposure levels below which no adverse effects 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*-octylphthalate. 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 1990a), 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.

The toxicity information presented in this chapter focuses on studies that have identified with reasonable certainty that their test material is di-*n*-octylphthalate or its monoester metabolite, mono-*n*-octylphthalate. Unfortunately, use of the nonspecific term "di-octylphthalate" has contributed to significant confusion and misinformation in the technical and governmental literature with respect to di-*n*-octylphthalate and its much more common isomer, di(2-ethylhexyl)phthalate. Although frequently interpreted as referring to di-*n*-octylphthalate, it is apparent that in almost all cases "di-octylphthalate" and "DOP" have in fact been used as synonyms for di(2-ethylhexyl)phthalate. Throughout this chapter whenever possible, an assessment of the level of certainty that the test compound was di-noctylphthalate will be made.

2.2.1 inhalation Exposure

No studies were located regarding the following health effects in humans or animals after inhalation exposure to di-*n*-octylphthalate:

2.2.1.1 Death

2.2.1.2 Systemic Effects

2.2.1.3 Immunological and Lymphoreticular Effects

2.2.1.4 Neurological Effects

2.2.1.5 Reproductive Effects

2.2.1.6 Developmental Effects

2.2.1.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

No studies were located regarding cancer in humans or animals after inhalation exposure to di-*n*-octylphthalate.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to di-*n*-octylphthalate.

In animals, the reported oral LD_{50} values are 53,700 mg/kg body weight for male albino rats (Dogra et al. 1987), 13,000 mg/kg for Swiss albino mice (Dogra et al. 1989), and >12,800 mg/kg for mice (Eastman Kodak Company 1978). Dosing was by gavage in these studies. No additional studies were located regarding death in animals after oral exposure to di-*n*-octylphthalate.

LOAEL values from each reliable study for death in each species and duration category are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.2.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, musculoskeletal, dermal, or ocular effects in humans or animals after oral exposure to di-*n*-octylphthalate. The systemic effects observed after oral exposure are discussed below. The highest NOAEL values and all LOAEL values from each reliable study for observed systemic effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-1.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in.humans after oral exposure to di-*n*-octylphthalate.

One study reported that feeding di-*n*-octylphthalate to groups of four male Wistar albino rats at average doses of 2,266 mg/kg/day for 3 days, 2,078 mg/kg/day for 10 days, or 1,096 mg/kg/day for 21 days did not result in any gross pathological changes in the pancreas (Mann et al. 1985). No

TABLE 2-1. Levels of Significant Exposure to Di-n-octylphthalate - Oral

Key [a] to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Death						
1	Rat Albino	NS				53700 M (LD_{50})	Dogra et al. 1987
2	Mouse Swiss	NS				13000 (LD_{50})	Dogra et al. 1989
	Systemic						
3	Rat Sprague-Dawley	4 d 1x/d (GO)	Bd Wt	2800 M			Foster et al. 1980
4	Rat Wistar	3 or 10 d ad lib (F)	Endocr		2000 M (decreased T_4 levels, damaged mitochondria, increased number and size of lysozomes, enlarged Golgi apparatus)		Hinton et al. 1986
5	Rat Sprague-Dawley	14 d 1x/d (GO)	Hepatic		1000 [b] M (17% increase relative liver weight, reduced 7-ethoxy-coumarin O-deethylase activity)		Lake et al. 1986

TABLE 2-1. Levels of Significant Exposure to Di-n-octylphthalate - Oral (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
6	Rat Wistar	3 d (F)	Gastro	2266 M			Mann et al. 1985
			Hepatic		2266 M (centrilobular glycogen loss, altered endoplasmic reticulum and bile canaliculi)		
			Renal	2266 M			
			Bd Wt	2266 M			
7	Rat Wistar	10 d (F)	Gastro	2078 M			Mann et al. 1985
			Hepatic			2078 M (necrosis, 19% increase liver weight; centrilobular glycogen loss, fat accumulation; altered endoplasmic reticulum and enzyme activities)	
			Renal	2078 M			
			Bd Wt	2078 M			
8	Rat Wistar	1 wk (F)	Hepatic		1000 M (increased liver weight)		Oishi and Hiraga 1980
			Renal	1000 M			
			Bd Wt	1000 M			
9	Mouse CD-1	14 d (F)	Other	7500	15000 (rough hair coat)		Heindel et al. 1989

TABLE 2-1. Levels of Significant Exposure to Di-n-octylphthalate - Oral (continued)

Key[a] to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
Immuno/Lymphor							
10	Rat Albino	5 d 1x/d (G)			2685 M (reduced antibody synthesis)		Dogra et al. 1987
Reproductive							
11	Rat Sprague-Dawley	4 d 1x/d (GO)		2800 M			Foster et al. 1980
12	Rat Wistar	10 d 1x/d (GO)		2800 M			Gray and Butterworth 1980
13	Rat Wistar	2 d 1x/d (GO)			2000 M (smooth endoplasmic reticulum vesiculation)		Jones et al. 1993
14	Rat Wistar	10 d (F)		2078 M			Mann et al. 1985
15	Rat Wistar	once (G)			2000 M (altered testicular mitochondrial function)		Oishi 1990

TABLE 2-1. Levels of Significant Exposure to Di-n-octylphthalate - Oral (continued)

Key [a] to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
16	Rat Wistar	1 wk (F)			1000 M (decreased testicular zinc levels)		Oishi and Hiraga 1980
	Developmental						
17	Mouse CD-1	Gd 6-13 1x/d (GO)				9780 F (reduced liveborn per litter)	Hardin et al. 1987
	INTERMEDIATE EXPOSURE						
	Systemic						
18	Rat Sprague-Dawley	10 wk ad lib (F)	Hepatic		500 M (mild fatty change, increased gamma glutamyl-transpeptidase + foci)		DeAngelo et al. 1986
19	Rat Wistar	21 d ad lib (F)	Endocr		2000 M (decreased T_4 levels, damaged mitochondria, increased number and size of lysozomes, enlarged Golgi apparatus)		Hinton et al. 1986

TABLE 2-1. Levels of Significant Exposure to Di-n-octylphthalate - Oral (continued)

Key [a] to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
20	Rat Wistar	21 d (F)	Gastro	1906 M			Mann et al. 1985
			Hepatic		1906 M (increased liver weight; centrilobular glycogen loss, marked fat accumulation, and some necrosis; altered endoplasmic reticulum and enzyme activities)		
			Renal	1906 M			
			Bd Wt	1906 M			

TABLE 2-1. Levels of Significant Exposure to Di-n-octylphthalate - Oral (continued)

Key to figure [a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
21	Rat Sprague-Dawley	13 wk ad lib (F)	Hemato	350.1 M			Poon et al. 1995
				402.9 F			
			Hepatic	36.8 M	350.1 M (cytoplasmic vacuolation, accentuation of zonation, increased ethoxyresorufin-O-deethylase activity)		
				40.8 [c] F	402.9 F (cytoplasmic vacuolation, accentuation of zonation, increased ethoxyresorufin-O-deethylase activity)		
			Renal	350.1 M			
				402.9 F			
			Endocr	36.8 M	350.1 M (reduced follicle size and colloid density in thyroid)		
				40.8 F	402.9 F (reduced follicle size and colloid density in thyroid)		
			Bd Wt	350.1 M			
				402.9 F			

TABLE 2-1. Levels of Significant Exposure to Di-n-octylphthalate - Oral (continued)

Key [a] to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
22	Mouse CD-1	85-105 d (F)	Hepatic		8640 B (increased absolute liver weight)		Heindel et al. 1989; Morrissey et al. 1989; NTP 1985
			Renal		8640 F (increased absolute kidney weight)		
			Bd Wt	8640 B			
23	Mouse CD-1	105 d (F)	Bd Wt	7460			Heindel et al. 1989; Morrissey et al. 1989; NTP 1985
Reproductive							
24	Rat Wistar	21 d (F)		1906 M			Mann et al. 1985
25	Rat Sprague-Dawley	13 wk ad lib (F)		350.1 M			Poon et al. 1995
26	Mouse CD-1	85-105 d (F)			8640 M (decreased seminal vesicle weight)		Heindel et al. 1989; Morrissey et al. 1989; NTP 1985
				8640 F			

TABLE 2-1. Levels of Significant Exposure to Di-n-octylphthalate - Oral (continued)

Key to figure [a]	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
27	Mouse CD-1	105 d (F)		7460			Heindel et al. 1989; Morrissey et al. 1989; NTP 1985
	Developmental						
28	Mouse CD-1	85-105 d (F)		8640			Heindel et al. 1989; Morrissey et al. 1989; NTP 1985
29	Mouse CD-1	105 d (F)		7460			Heindel et al. 1989; Morrissey et al. 1989; NTP 1985

[a]The number corresponds to entries in Figure 2-1.

[b]Used to derive an acute-duration oral minimal risk level (MRL) of 3 mg/kg/day; dose divided by an uncertainty factor of 300 (3 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

[c]Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.4 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ad lib = ad libitum; B = both sexes; Bd Wt = body weight; d = day(s); Endocr = endocrine; F = female; (F) = feed; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day(s); (GO) = gavage, in oil; Hemato = hematological; LD_{50} = median lethal dose; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; wk = week(s); x = time(s)

Figure 2-1. Levels of Significant Exposure to Di-*n*-octylphthalate - Oral

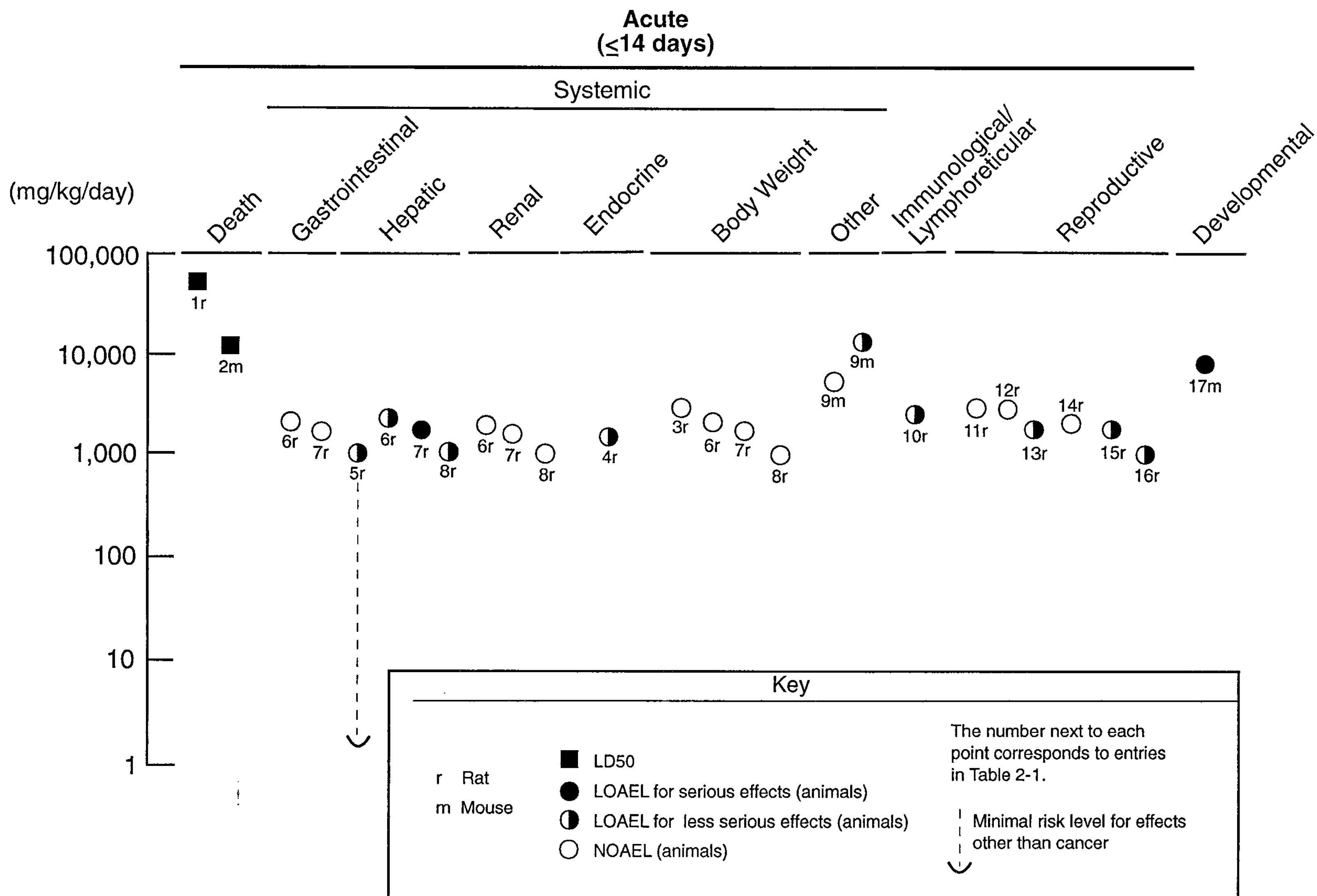
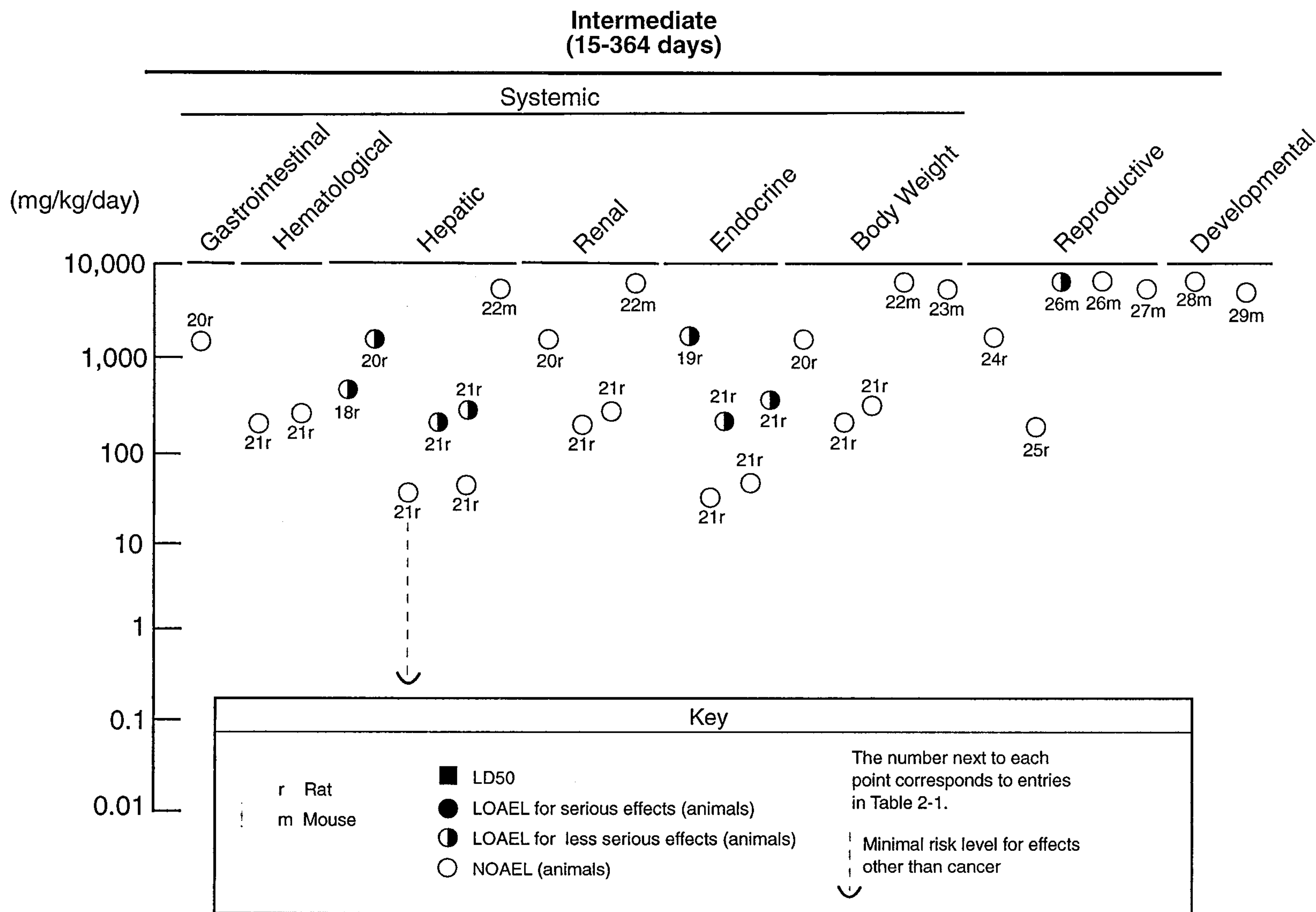


Figure 2-1. Levels of Significant Exposure to Di-*n*-octylphthlate - Oral (continued)



analytical data were reported, but it is reasonably certain that the test compound was di-*n*-octylphthalate (purity was reported to be 99.5%).

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to di-*n*-octylphthalate.

One study in Sprague-Dawley rats (10/sex/group) reported that feeding di-*n*-octylphthalate at concentrations up to 5,000 ppm (350.1 or 402.9 mg/kg/day in males or females, respectively) for 13 weeks did not affect hematological parameters (Poon et al. 1995).

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to di-*n*-octylphthalate.

Several studies were located that reported a variety of hepatic effects in rats and mice usually after large oral doses were administered for acute or intermediate durations. These include effects of di-*n*-octylphthalate on liver appearance, structure, and function.

Gross Appearance and Organ Mass. Upon dietary exposure of groups of four male Wistar albino rats to 1,906-2,266 mg/kg/day of di-*n*-octylphthalate for 3-21 days, livers were reported to be pale and greasy in appearance (Mann et al. 1985). When compared with control values, no change in liver weight was observed after 3 days of treatment, although small but significant ($p < 0.01$) increases in relative liver weight (liver weight as a percentage of body weight) were noted after 10 days (4.7% versus 4.0%, or a 19% change from control) and 21 days (4.1% versus 3.2%, or a 28% change from control) of treatment. Similarly, absolute (15%) or relative (16%) liver weights were increased significantly in 10 male Wistar rats after 7 days of dietary exposure to 1,000 mg/kg/day (Oishi and Hiraga 1980). Relative liver weight was significantly increased in 6 male Sprague-Dawley rats after 14 days of exposure by gavage to 1,000 or 2,000 mg/kg/day (Lake et al. 1984, 1986). No changes in absolute or relative liver weights were noted after Sprague-Dawley rats (10/sex/group) were fed concentrations of up to 5,000 ppm (350.1 or 402.9 mg/kg/day in males and females, respectively) in the diet for 13 weeks (Poon et al. 1995).

Male rats, initiated with a single intraperitoneal dose of the carcinogen diethylnitrosamine and then partially hepatectomized, did not experience any liver weight gain after 10 weeks of dietary exposure

to 500 mg/kg/day of di-*n*-octylphthalate (DeAngelo et al. 1986). When exposure was extended to 26 weeks, small increases in absolute liver weight that were not significant ($p < 0.05$) were observed at di-*n*-octylphthalate doses of 250 mg/kg/day (2% increase) and 500 mg/kg/day (8% increase). However, when combined with diminished body weight gains, relative liver weight gains were increased by 5-16% when compared with control values (Carter et al. 1992). These results should be considered independently of the other studies discussed because the animals were surgically altered and chemically treated with diethylnitrosamine.

Dietary exposure for 85-105 days (including lactation during exposure of the dams) to 8,640 mg/kg/day of di-*n*-octylphthalate also induced significant ($p < 0.05$) absolute liver weight gains in male and female CD-l mice (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985). All three of these papers reported the same study; the dose specified here is from the Morrissey et al. (1989) report. A l-week dietary exposure to 1,000 mg/kg/day of mono-*n*-octylphthalate was also reported to induce liver enlargement in seven male Wistar rats (Oishi and Hiraga 1982). Whether those modest liver weight increases resulted primarily from hyperplasia or from hypertrophy is not clear. However, the absence of increased hepatic mitotic activity in two of four rats after di-*n*-octylphthalate exposure in the Mann et al. (1985) study noted above suggests that significant hyperplasia may not be a factor.

Morphology, Histopathology, and Biochemistry. Aside from the pale, greasy appearance noted above, livers from male Wistar rats exposed for 3 days to 2,266 mg/kg/day of di-*n*-octylphthalate in the diet displayed a loss of centrilobular glycogen (Mann et al. 1985). Proliferation and dilation of the smooth endoplasmic reticulum accompanied by some loss of rough endoplasmic reticulum were noted, as were shortened microvilli in some bile canaliculi. No significant changes were noted in parameters associated with hepatic peroxisomal activity (cyanide-insensitive palmitoyl CoA oxidase, α-glycero-phosphate dehydrogenase, and total or peroxisomal catalase activities), plasma membrane integrity (plasma membrane 5'-nucleotidase activity), mitochondrial respiration (succinate dehydrogenase activity), endoplasmic reticular function (glucose-6-phosphatase activity and level of cytochrome P-450), or in the level of nonenzymic reductants. When exposure time was increased to 10 or 21 days (average doses of 2,078 or 1,906 mg/kg/day di-*n*-octylphthalate, respectively), the centrilobular reduction in glycogen became more severe and was associated with fat accumulation and some necrosis. However, the almost total loss of liver glycogen that was observed when using similar doses of di(2-ethylhexyl)phthalate did not occur. Lipid droplets were observed in hepatocytes, along with a possible small increase in the number of peroxisomes; the endoplasmic reticulum morphology

alterations remained apparent. Small but significant ($p < 0.01$ or 0.05) increases were found in hepatic cyanide-insensitive palmitoyl CoA oxidase and peroxisomal catalase activities but not in α-glycerophosphate dehydrogenase or total catalase activities. Significant reductions were noted in 5'-nucleotidase, succinate dehydrogenase, and glucose-6-phosphatase activities compared to the controls. In contrast to di-*n*-octylphthalate treatment, similar doses of di(2-ethylhexyl)phthalate resulted in a dark, enlarged liver, and an initial burst of mitosis was noted at day 3 in two out of four rats. A lesser degree of early centrilobular glycogen loss was noted with di(ethylhexyl)phthalate, but almost total liver glycogen loss occurred after 21 days along with periportal rather than centrilobular fat accumulation, no centrilobular necrosis, pronounced peroxisome proliferation, greater smooth endoplasmic reticulum proliferation, mitochondrial matrix changes, and larger reductions in 5'-nucleotidase, glucose-6-phosphatase, and nonenzymic reductant activities.

Sprague-Dawley rats (10/sex/group) fed 0, 5, 50, 500, or 5,000 ppm of di-*n*-octylphthalate in the diet, corresponding to intakes of 0, 0.4, 3.5, 36.8, and 350.1 mg/kg/day (males) or 0, 0.4, 4.1, 40.8, and 402.9 mg/kg/day (females), for 13 weeks showed significant increases in hepatic ethoxyresorufin-*O*-demethylase activities at the highest dose (Poon et al. 1995). There were histopathologic changes in hepatic architecture noted at 5,000 ppm, however, which included a moderate degree of accentuation of zonation in all animals of both sexes and mild-to-moderate perivenous cytoplasmic vacuolation in 9/10 males and 5/10 females (Poon et al. 1995). These effects were not observed at 500 ppm, which represents the NOAEL. There was no visual increase in peroxisomes noted at any dose level and no changes in activities of either amino-*N*-demethylase or aniline hydrolase.

Rats exposed to dietary concentrations of mono-*n*-octylphthalate equivalent to 1,000 mg/kg/day exhibited a variety of significant alterations in serum lipid composition, reflecting a possible effect on the hepatic metabolism of lipids (Oishi and Hiraga 1982). With respect to control values, serum concentrations of phospholipids and nonesterified fatty acids were increased, while those of triglycerides and total cholesterol were decreased. Levels of free cholesterol, lipoperoxides, and 1ecithin:cholesterol acyltransferase activity were not significantly affected. Mono-*n*-octylphthalate exposure increased palmitic acid content while decreasing stearic acid content in serum phospholipid; increased oleic acid content in serum phospholipid, cholesteryl ester, and triglyceride; decreased linoleic acid content in serum triglyceride; and decreased arachidonic acid content in serum phospholipid. Several other monophthalates were also evaluated, with mono-2-ethylhexylphthalate being generally somewhat more potent than mono-*n*-octylphthalate. The compositional effects of

mono-2-ethylhexylphthalate on serum cholesteryl ester were broader and more pronounced than those of mono-*n*-octylphthalate, while the reverse was true for serum triglyceride (mono-2-ethylhexylphthalate was without significant effect). Such effects could result in part from altered gastrointestinal digestion and absorption of dietary fat, but as suggested by the previously noted accumulation of fat in the liver (Mann et al. 1985), they may reflect altered hepatic metabolism of fatty acids and cholesterol. The toxicological significance of these alterations in lipid metabolism is not known. The study authors noted that the general trend of these serum lipid changes and the attendant increases in liver size are significantly similar to the effects observed after exposure to di(2-ethylhexyl)phthalate (see also ATSDR 1992), thus implicating the monoesters or subsequent metabolites, rather than the diesters, as the active compounds inducing these effects.

Treating male Sprague-Dawley rats with di-*n*-octylphthalate (2,000 mg/kg/day) or mono-*n*-octylphthalate (750 and 1,000 mg/kg/day) by gavage for 14 days (5 rats/dose) did not induce hepatic peroxisome proliferation (Lake et al. 1984). Similar treatment with 1,000 mg/kg/day di-*n*-octylphthalate or 715 mg/kg/day mono-*n*-octylphthalate did not significantly increase (cyanide-insensitive palmitoyl-CoA oxidase and heat-liable enoyl-CoA hydratase) or reduce (*O*-amino acid oxidase) hepatic enzyme activities associated with peroxisome proliferation (Lake et al. 1984, 1986). Both di-*n*-octylphthalate and mono-*n*-octylphthalate significantly reduced ($p < 0.05$) hepatic microsomal-7-ethoxyresorutin *O*-deethylase activity, and di-*n*-octylphthalate reduced 7-ethoxycoumarin *O*-deethylase activity, but neither di-*n*-octylphthalate nor mono-*n*-octylphthalate significantly affected other mixed function oxidase activities (ethylmorphine n-demethylase, lauric acid 11-and 12-hydroxylases), microsomal cytochrome P-450 content, microsomal hemoprotein spectral properties, or sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of hepatic whole homogenates and microsomes. Again, these results were substantially different from those obtained with doses of 1,000 mg/kg di(2-ethylhexyl)phthalate and 500 mg/kg of the hypolipidemic drug clofibrate.

Two additional rat studies designed to investigate the reproductive effects of di-*n*-octylphthalate exposure provide another observation on the hepatic effects of di-*n*-octylphthalate. Contrary to what is observed with di(2-ethylhexyl)phthalate, the concentration in liver of the essential element zinc was found not to be significantly reduced after 4 days of gavage exposure to 2,800 mg/kg/day of di-*n*-octylphthalate (Foster et al. 1980), or 7 days of dietary exposure to 1,000 mg/kg/day of di-*n*-octylphthalate (Oishi and Hiraga 1980).

The only oral study located that evaluated the presence of hepatic effects in a species other than rats was a reproductive-developmental toxicity study conducted according to the NTP Continuous Breeding Protocol (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985). F_1 generation CD-l mice (offspring from the fifth litter of a treated F_0 generation) were exposed via lactation to di-*n*-octylphthalate (from dams exposed to approximately 7,460 mg/kg/day) and then via feed after weaning until they were 85-105 days old. The average di-*n*-octylphthalate dose over this period was calculated by the study authors to be 8,640 mg/kg/day. As might be expected from prior rat data, statistically significant increases in absolute liver weight (24%) were noted. The study authors reported that no gross morphological or histopathological alterations were observed in the treated livers. This is despite substantially higher doses and longer treatment times than were used in the rat studies and despite a fourfold lower acute oral LD_{50} reported for mice than for rats (Dogra et al. 1987, 1989). As has been noted for di(2-ethylhexyl)phthalate (ATSDR 1992), the degree and nature of hepatotoxicity resulting from exposure to di-*n*-octylphthalate may vary considerably with species and also with dosage procedures. Dogra et al. (1987, 1989) employed oral gavage with rats and mice, whereas the NTP study employed dietary feeding with mice.

In summary, the liver appears to be a primary target organ for the toxic effects of acute- and intermediate-duration high-dose exposure to di-*n*-octylphthalate (and mono-*n*-octylphthalate), at least in the rat and mouse. Unlike its branched-chain isomer di(2-ethylhexyl)phthalate, di-*n*-octylphthalate presents a liver toxicity profile only weakly suggestive of the hypolipidemic peroxisome proliferators (e.g., clofibrate). Instead, the liver changes associated with exposure to di-*n*-octylphthalate are characterized by marked centrilobular accumulation of fat and loss of glycogen, accompanied by reduced glucose-6-phosphatase, cytoplasmic vacuolation, accentuation of zonation, and some centrilobular necrosis. However, although these effects have been noted in two studies (Mann et al. 1985; Poon et al. 1995), they were not seen in a multigeneration study in mice by NTP (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985) or in other studies in rats (Lake et al. 1984, 1986; Oishi and Hiraga 1980). It should be mentioned here that di-*n*-octylphthalate has been shown to induce additional liver effects associated with preneoplastic alteration; these are discussed below under "Cancer."

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to di-*n*-octylphthalate.

Few animal studies were located that report any observations on the renal toxicity of di-*n*-octylphthalate. In a study examining the effects of di-*n*-octylphthalate on testicular function, rats that had received 2,800 mg/kg/day by gavage for 4 days were observed to have a small (9%) but not statistically significant reduction in kidney zinc concentration when compared with controls (Foster et al. 1980). Total urinary excretion of zinc was reduced to 85% of controls, but again this was not significant. When expressed as a percentage of zinc excretion on day 0 (i.e., prior to di-noctylphthalate administration), the 4-day urinary excretion profile of zinc in the di-*n*-octylphthalate-treated rats was virtually identical to that of controls. No effect on kidney weight was observed. In contrast, these parameters were generally elevated after treatment with n-alkylphthalates that induced testicular pathology (di-*n*-butylphthalate, di-*n*-pentylphthalate, and di-*n*-hexylphthalate; see "Reproductive Effects," below). These findings are supported by another study in rats in which dietary exposure of male Wistar rats for 1 week to 1,000 mg/kg/day of di-*n*-octylphthalate did not affect the concentration of zinc in the kidney (phthalic acid slightly increased it) nor significantly ($p < 0.05$) reduce kidney weight as did di(2-ethylhexyl)phthalate (Oishi and Hiraga 1980). No gross pathological changes were observed in the kidneys of male Wistar rats following 3-, 10-, or 21-day exposure to 2% di-*n*-octylphthalate in the diet (2,266, 2,078, or 1,906 mg/kg/day, respectively) (Mann et al. 1985). No effects on absolute or relative kidney weight were noted when Sprague-Dawley rats of both sexes were fed di-*n*-octylphthalate for 13 weeks at concentrations up to 5,000 ppm (350.1 or 402.9 mg/kg/day in males and females, respectively) (Poon et al. 1995).

One reproductive-developmental toxicity study in CD-l mice also examined the renal effects of di-*n*-octylphthalate exposure (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985). Following the NTP Continuous Breeding Protocol, F_1 mice taken from the last litter of treated F_1 parental mice (7,460 mg/kg/day for 105 days, including the mating period) were exposed for 85-105 days to an average calculated di-*n*-octylphthalate dose of 8,640 mg/kg/day (first via lactation, then feed). Although no gross morphological or histopathological changes in the kidney were noted, absolute kidney weight was significantly ($p < 0.05$) elevated in female (1l%), but not male, mice.

Despite the absence of data on organ function, these limited results suggest that acute or intermediate oral exposure to even very high doses of di-*n*-octylphthalate is not likely to result in substantial renal toxicity.

Endocrine Effects. No studies were located regarding endocrine effects in humans after oral exposure to di-*n*-octylphthalate.

There is some evidence in animals that suggests that oral administration of di-*n*-octylphthalate may affect the thyroid gland. Serum from four male Wistar rats that were fed di-*n*-octylphthalate at a concentration of 2% in the diet (approximately 2,000 mg/kg/day) in a previous study (Mann et al. 1985) was reassayed (Hinton et al. 1986). After 3, 10, and 21 days of treatment, significant decreases in thyroxine (T_4) levels were noted compared to the controls. T_4 levels were 47%, 59%, and 76% of control values after 3, 10, and 21 days of treatment, respectively. No significant effects on triiodothyronine (T_3) levels were noted compared to the controls (Hinton et al. 1986). In addition, marked ultrastructural changes were noted in the thyroids of these animals, including increases in the numbers and size of lysosomes, enlargement of the Golgi apparatus, and apparent damage to the mitochondria (Hinton et al. 1986). This study was limited, however, in that only one concentration was tested.

Groups of 10 male and 10 female Sprague-Dawley rats that were administered di-*n*-octylphthalate in the diet for 13 weeks at concentrations of 0, 5, 50, 500 and 5,000 ppm, corresponding to intakes of 0, 0.4, 3.5, 36.8, and 350.1 mg/kg/day (males) and 0, 0.4, 4.1, 40.8, and 402.9 mg/kg/day (females) showed reductions in the size of thyroid follicles and mild decreases in colloid density at 5,000 ppm (Poon et al. 1995). Changes in these parameters was also noted at 500 ppm, but it is not whether this concentration represents a LOAEL, because statistical analysis of the data was not performed.

Body Weight Effects. No studies were located regarding body weight effects in humans after oral exposure to di-*n*-octylphthalate.

Data from various studies consistently indicate that acute or intermediate oral exposures to high doses (l,000-15,000 mg/kg/day) of di-*n*-octylphthalate do not adversely affect either body weight gain or food consumption in rats or mice (Carter et al. 1992; Heindel et al. 1989; Mann et al. 1985; Morrissey

et al. 1989; NTP 1985; Oishi and Hiraga 1980, 1982). In male Wistar rats receiving di-noctylphthalate at a concentration of 2% in the diet (approximately 2,000 mg/kg/day), food intake was significantly ($p < 0.01$) increased after 3 days when compared with controls (13.7 versus 19.5 g/kg/rat), and body weight was significantly ($p < 0.05$) increased after 10 days relative to controls (204 versus 220 g/rat). By days 11-21, however, the values for both parameters returned to control levels (Mann et al. 1985). Male rats subjected to a single intraperitoneal dose of diethylnitrosamine, partial hepatectomy, and 26 weeks of dietary exposure to 250 or 500 mg/kg/day of di-*n*-octylphthalate apparently experienced small reductions (3-7%) in body weight gain (Carter et al. 1992). Male Wistar rats fed 1,000 mg/kg/day of the monoester mono-*n*-octylphthalate for 1 week were reported to have depressed body weight gains during the first 2 days and reduced body weights at the experiment's end, but quantitative data were not provided (Oishi and Hiraga 1982).

Other Systemic Effects. No studies were located regarding other systemic effects in humans after oral exposure to di-*n*-octylphthalate.

After a 14-day exposure of CD-l mice to 1,800, 3,600, 7,500, or 15,000 mg/kg/day, a rough hair coat was noted in four to six out of eight animals of both sexes at 15,000 mg/kg/day (Heindel et al. 1989; NTP 1985).

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans after oral exposure to di-*n*-octylphthalate.

Limited data suggest that di-*n*-octylphthalate can exert immunotoxic effects in rats and mice after acute oral exposure to relatively high doses. Male rats exposed by gavage once per day for 5 days to 0, 2,685, 5,370, or 10,740 mg/kg/day of di-*n*-octylphthalate (acute LD_{50} was 53,700 mg/kg) did not display any signs of overt toxicity, but did exhibit a depletion of cells in the periarteriolar lymphoid sheet of the spleen that the study authors noted as dose dependent, although doses at which these effects were observed were not identified (Dogra et al. 1987). Dose-dependent cellularity changes in the thymus resulted in a loss of distinction between the cortex and the medulla, and germinal center activity was diminished in regional and peripheral lymph nodes. In response to the intraperitoneal injection of sheep red blood cells (SRBC), the number of IgM-producing spleen cells was significantly

($p < 0.001$) reduced to approximately 10% or 5% of the control value in rats exposed to the mid- and high-doses, respectively. There was a concomitant, dose-dependent, 1,000-fold reduction in serum anti-SRBC antibodies. Phagocytic and metabolic activities of peritoneal exudate cells were also reduced by up to 30-40%. When challenged by subcutaneous inoculation of 1,000 larvae from the parasite *Nippostrangylus brasiliensis,* 10-day worm counts were indicated to be significantly elevated by mid- and high-dose treatments (19% and 30%, respectively) when compared with controls. Finally, mortality was increased from 2- to 2.5-fold in rats treated with 10,740 mg/kg/day di-*n*-octylphthalate (versus controls) when they were subsequently challenged with intravascular injections of 125 or 250 pg of lipopolysaccharide endotoxin from *Escherichia coli.*

The immune system of the mouse may also be susceptible to the effects of acute oral exposures to di-*n*-octylphthalate (Dogra et al. 1989). Three-month-old Swiss albino mice were exposed to di-*n*-octylphthalate by gavage for 5 days at 0, 650, or 2,600 mg/kg/day (acute LD_{50} was 13,000 mg/kg). Mice were subsequently exposed by intraperitoneal injection to either encephalomyocarditis virus or the malarial protozoan, *Plasmodium berghei.* Maximum mortality levels were reached 8-10 days after viral infection and were 20% (0 mg/kg/day), 40% (650 mg/kg/day), and 70% (2,600 mg/kg/day). Malarial lethality reached plateau levels 4-l 1 days postinfection of approximately 20% (0 mg/kg/day), 25% (650 mg/kg/day), and 70% (2,600 mg/kg/day), then increased to 55%, 70%, and 85%, respectively, by postinfection day 19. Respective mean survival times were calculated to be 13.50, 12.15, and 6.25 days. During the first 14 days after protozoa1 infection, the percentage of mouse erythrocytes infected with the parasite in the high-dose group was consistently and significantly ($p < 0.01$ or 0.05) higher than in the control group. Significant increases were generally not observed in the low-dose group.

Both of these studies did not contain positive controls, and both omitted experimental details and much quantitative data. In addition, no proof of compound identity was provided and the dose levels might be considered high enough to risk inducing overt or generalized systemic toxicity. However, the authors indicated that no signs of gross or other organ toxicity were observed, and previously discussed studies appear, in general, to indicate only adverse hepatic effects following acute oral exposure to di-*n*-octylphthalate. In combination, these studies suggest that acute oral exposure to high doses of di-*n*-octylphthalate, at least in the rat and the mouse, may result in compromised immune responses to bacterial, viral, protozoan, or other parasitic infection.

The highest NOAEL values and all LOAEL values from each reliable study for immunological effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to di-*n*-octylphthalate.

Limited data from one study on CD-l mice indicate that acute and intermediate dietary exposures to di-*n*-octylphthalate at doses of up to 15,000 mg/kg/day produced virtually no effect on clinical signs of toxicity (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985). No clinical signs of neurotoxicity were noted after a 14-day exposure to 1,800, 3,600, 7,500, or 15,000 mg/kg/day; rough hair coats were noted at the highest dose. No clinical signs of neurotoxicity were observed in the mice following exposure to 1,820, 3,520, or 7,460 mg/kg/day for 105 days, nor in the offspring of the high-dose group that were exposed via lactation followed by feed for 85-105 days to an average di-*n*-octylphthalate dose of 8,640 mg/kg/day.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to di-*n*-octylphthalate.

The results of several acute- or intermediate-duration rodent studies indicate that the potential of di-*n*-octylphthalate exposure to cause adverse reproductive effects is very low. When male rats were exposed by gavage to 2,800 mg/kg/day of di-*n*-octylphthalate for either 4 or 10 days, no testicular atrophy (weight loss or histological lesions), testicular zinc loss, or weight loss of the prostate or seminal vesicles were observed (Foster et al. 1980; Gray and Butterworth 1980). Such effects were induced by di-*n*-butylphthalate, di-*n*-pentylphthalate, di-*n*-hexylphthalate, and di(2-ethylhexyl)phthalate, but not by the methyl, ethyl, *n*-propyl, or *n*-heptylphthalate diesters. No adverse effects on testis weight or histopathology were found in male rats exposed to up to 5,000 ppm (402.9 mg/kg/day) of di-*n*-octylphthalate in the diet for 13 weeks (Poon et al. 1995). Similarly, no effect on testis weight, gross morphology, or histopathology was found in male rats receiving dietary exposure to approximately 2,000 mg/kg/day for 10 or 21 days (Mann et al. 1985). In another study in which male

rats were fed a diet containing 1,000 mg/kg/day of di-*n*-octylphthalate for 1 week, the absence of effect on absolute and relative testis weight was confirmed, and no effect was found on testicular concentrations of testosterone or dihydrotestosterone; however, a significant (15%, $p < 0.05$) reduction in testicular zinc concentration was observed (Oishi and Hiraga 1980). One or more of these parameters was also altered by di-o-butylphthalate, diisobutylphthalate (DiBP), dimethyl phthalate (DMP), diethyl phthalate (DEP), di(2-ethylhexyl)phthalate, and phthalic acid (PA). These data are insufficient to clarify the precise relationship among testicular atrophy, high testosterone, and low zinc, but the study authors speculated that testicular atrophy may depend on phthalate induction of elevated levels of testosterone in the testis, accompanied by reduced zinc levels in both the testis and the liver.

Some small, but statistically significant ($p < 0.05$), changes in testicular mitochondrial respiratory functions were observed in 35-day-old male rats 6 hours after they had received a single oral dose of 2,000 mg/kg di-*n*-octylphthalate by gavage (Oishi 1990). Oxygen consumption of mitochondrial preparations from the testis during state 3 respiration (succinate respiration in the presence of adenosine diphosphate [ADP]; phosphorylation) was reduced by 20% when compared with untreated control values, and the respiratory control ratio of state 3 to state 4 respiration ("resting" succinate respiration in the absence of ADP), which is a measure of respiration dependency on ADP, was also slightly reduced by 8%. Pyruvate and lactate concentrations were not changed, nor was phosphorylative activity (the state 3 ratio of ADP to oxygen consumption). These effects were generally less extensive than those induced by di(2-ethylhexyl)phthalate treatment. Routine histopathological examination showed no changes in the seminiferous tubule structure in 6-8 week old Wistar rats after single gavage doses of 2,000 mg/kg/day on each of 2 consecutive days (Jones et al. 1993). However, electron microscopic examination revealed vesiculation of the smooth endoplasmic reticulum and increased stacking into parallel cistemae in some Leydig cells, but no mitochondrial swelling or degeneration. This study is limited, however, because the tabular listing of effects noted for di-*n*-octylphthalate in this paper shows no vesiculation of smooth endoplasmic reticulum (Jones et al. 1993).

Finally, one mouse reproductive-developmental toxicity study performed according to the NTP Continuous Breeding Protocol was reported in three papers (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985). Dietary exposure of F_0 generation male and female CD-l mice for 105 days to doses of 0, 1,820, 3,620, or 7,460 mg/kg/day of di-*n*-octylphthalate failed to cause any reduction in fertility index (percent fertile pairs) or number of litters/pair. In contrast, at least one of these parameters was

significantly reduced after exposure to di-*n*-propylphthalate or di-*n*-pentylphthalate (8,600 mg/kg/day of di-*n*-propylphthalate and 2,160 mg/kg/day of di-*n*-pentylphthalate completely inhibited fertility). Similarly, F_1 generation mice exposed via lactation and then feed to an average di-*n*-octylphthalate dose of 8,640 mg/kg/day for 85-105 days exhibited a statistically significant ($p < 0.05$) reduction in seminal vesicle weight, but no significant changes were noted for testis, cauda epididymis, or prostate weights, or for sperm concentration, percent mobile sperm, or percent abnormal sperm. In female mice, estrous cycle length was not altered, and no reproductive tract organ weight or histopathological changes were observed. These findings confirm that di-*n*-octylphthalate has a low potential for inducing reproductive toxicity following oral exposure.

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2-l.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to di-*n*-octylphthalate.

In a preliminary developmental toxicity screening study, female CD-l mice received 9,780 mg/kg/day of di-*n*-octylphthalate by gavage once per day during gestation days 6-13 (Hardin et al. 1987; NIOSH 1983). The following data were recorded: the number of pups born alive; total litter weight, pup survival, and litter weight gain immediately after birth and on postpartum day 3; maternal survival and weight gain from gestation day 6 to postpartum day 3; and the number of viable litters. The test material given was undiluted di-*n*-octylphthalate; the maximum feasible dose was considered to have been administered because an oral LD_{10} (the preferred dose) could not be established due to a lack of toxicity. The di-*n*-octylphthalate group varied significantly ($p < 0.05$) from its concurrent corn oil control group only in reduced number of livebom pups per litter (10.2 ± 2.8 versus 11.5 ± 1.7; 11% less than control) and reduced pup weight gain ($0.6 \pm 0.l$ g versus 0.7 ± 0.2 g; 14% change from control). However, the concurrent control values for these two parameters (especially for the number of liveborn pups per litter) were unusually high, and the authors reported that these two parameters were generally higher than those of other control groups from the same study, thus casting additional uncertainty on the biological relevance of these statistically significant changes.

The only other oral study located that examined developmental effects was the NTP Continuous Breeding Protocol study previously discussed in Section 2.2.2.5 (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985). F_0 generation CD-l mice were exposed to di-*n*-octylphthalate doses of 0, 1,820, 3,620, or 7,460 mg/kg/day for 105 days. F_1 generation mice (from the F_0 high-dose group) were subsequently exposed via lactation and then via feed to an average di-*n*-octylphthalate dose of 8,640 mg/kg/day for 85-105 days. In neither case were any significant effects noted for the number of live pups per litter, the proportion of pups born alive, pup sex ratio, or the live pup mean weight.

The combined results of these two studies indicate that di-*n*-octylphthalate probably has a very low potential to induce developmental toxicity, especially in view of the very high doses that were evaluated. The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after oral exposure to di-*n*-octylphthalate.

Genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to di-*n*-octylphthalate.

Two studies were located in which rats received di-*n*-octylphthalate dietary exposures of 250 or 500 mg/kg/day for either 10 or 26 weeks (Carter et al. 1992; DeAngelo et al. 1986). Five male rats were first initiated with a single subcarcinogenic intraperitoneal dose of diethylnitrosarnine (30 mg/kg), followed by partial hepatectomy. Di-*n*-octylphthalate caused substantial increases in gamma-glutamyltranspeptidase (GGT) positive liver foci when compared with the controls (e.g., from 3.5 to 20.8 foci/cm^2) or in hepatic levels of marker enzymes for altered cellular foci (GGT and glutathione *S*-transferase [GST]). Only a slight increase (threefold) was observed for carnitine acetyltransferase (CAT) activity, a marker for peroxisome proliferation. In contrast, while inducing CAT activity

37-40-fold, di(2-ethylhexyl)phthalate and mono(ethylhexyl)phthalate actually inhibited the foci-associated parameters. These results, although not definitive, suggest that di-*n*-octylphthalate may promote preneoplastic lesions in the rat liver, probably by a mechanism that does not rely on peroxisome proliferation.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans or animals following dermal exposure to di-n-octylphthalate.

2.2.3.2 Systemic Effects

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

Dermal and ocular effects observed after dermal exposure are discussed below.

Dermal Effects. No studies were located regarding dermal effects in humans following dermal exposure to di-*n*-octylphthalate.

In a toxicity summary submitted by Eastman Kodak Company (1978), di-*n*-octylphthalate was reported to be a slight skin irritant when applied to the depilatated skin of guinea pigs. However, di-n-octylphthalate was not a skin sensitizer in guinea pigs.

Ocular Effects. No studies were located regarding ocular effects in humans following dermal exposure to di-*n*-octylphthalate.

In a toxicity summary submitted by Eastman Kodak Company (1978), ocular administration of di-n-octylphthalate resulted in slight conjunctival irritation and no cornea1 damage. No further details were provided.

2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans following dermal exposure to di-*n*-octylphthalate.

Di-*n*-octylphthalate was negative in a skin sensitization test in guinea pigs (Eastman Kodak Company 1978). No further details were provided in this summary report.

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

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals following dermal exposure to di-n-octylphthalate.

2.3 TOXICOKINETICS

No studies were located regarding the toxicokinetics of di-*n*-octylphthalate in humans or animals following inhalation or dermal exposure. Information on the toxicokinetics of di-*n*-octylphthalate in humans following oral exposure is not available. There are studies that provide indirect evidence for the oral absorption of di-*n*-octylphthalate in animals (Albro and Moore 1974; Oishi 1990; Poon et al. 1995); however, quantitative information is lacking on the rate and extent of absorption following oral exposure to di-*n*-octylphthalate. Information on the distribution of di-*n*-octylphthalate is limited to oral studies in rats by Oishi (1990), which reported the identification of mono-*n*-octylphthalate in blood and

testes within 1-24 hours (plasma peak at 3 hours, testes peak at 6 hours) after dosing, and by Poon et al. (1995), which reported di-*n*-octylphthalate residues in liver and adipose tissue. The metabolism of di-*n*-octylphthalate following acute exposure has been studied in animals *in vivo* and *in vitro* (Albro and Moore 1974; Brodsky et al. 1986; Lake et al. 1977), and the data indicate that, like most phthalate esters, di-*n*-octylphthalate can be hydrolyzed at one or both ester linkages to produce the monoester as well as phthalic acid (minor metabolite). As with other phthalates, subsequent oxidation of the remaining arylester to short-chained carboxyls, alcohols, and ketones has been demonstrated. Although one study seems to indicate that urine is the major elimination route of di-*n*-octylphthalate metabolites following oral exposure (Albro and Moore 1974), no quantitative information on the rate and extent of excretion is available. No information is available on the mechanism of action of di-n-octylphthalate with respect to its absorption, distribution, metabolism, or excretion.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

No studies were located regarding the absorption of di-*n*-octylphthalate in humans or animals following inhalation exposure.

2.3.1.2 Oral Exposure

No studies were located regarding the absorption of di-*n*-octylphthalate in humans following oral exposure.

Evidence of oral absorption in rats is demonstrated in the studies by Albro and Moore (1974), Oishi (1990), and Poon et al. (1995). Forty-eight hours after a gavage dose of di-*n*-octylphthalate, metabolites were detected in the urine. The major metabolite (60% of the metabolites in urine) was derived from the monoester (Albro and Moore 1974). The mono-*n*-octylphthalate metabolite was found in the blood and testes of rats from l-24 hours after oral dosing with peak levels reported at 3 hours (for blood) and 6 hours (for testes) (Oishi 1990). Di-*n*-octylphthalate was found in the liver and adipose tissue of rats after they were fed this compound for 13 weeks in dietary concentrations up to 5,000 ppm, indicating its absorption (Poon et al. 1995). Although there are insufficient quantitative data for estimating the oral absorption rate, di-*n*-octylphthalate appears to be absorbed readily;

however, it may have to be converted to mono-*n*-octylphthalate for intestinal absorption to occur (Lake et al. 1977).

2.3.1.3 Dermal Exposure

No studies were located regarding the absorption of di-*n*-octylphthalate in humans or animals following dermal exposure.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding the distribution of di-*n*-octylphthalate in humans or animals following inhalation exposure.

2.3.2.2 Oral Exposure

No studies were located regarding the distribution of di-*n*-octylphthalate in humans following oral exposure.

Following a single oral dose of 2,000 mg/kg of di-*n*-octylphthalate in rats, mono-*n*-octylphthalate was detected in blood with peak levels observed at 3 hours and in the testes with peak levels observed at 6 hours (Oishi 1990). The biological half-life and mean residence time of mono-*n*-octylphthalate in blood were 3.3 and 5.4 hours, respectively. After 13 weeks of oral exposure of rats to di-*n*-octylphthalate in the diet at concentrations up to 5,000 ppm (350 and 403 mg/kg/day in males and females, respectively), the livers contained di-*n*-octylphthalate residues that were either below or just slightly above the detection limit (<3 ppm) (Poon et al. 1995). The adipose tissue of rats fed 5,000 ppm showed di-*n*-octylphthalate residue levels of 15 ppm (males) and 25 ppm (females). This study is limited in that it did not analyze tissues for the presence of metabolites.

2.3.2.3 Dermal Exposure

No studies were located regarding the distribution of di-*n*-octylphthalate in humans or animals following dermal exposure.

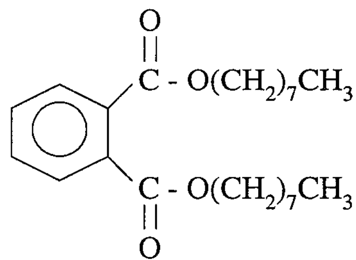
2.3.3 Metabolism

Di-*n*-octylphthalate is readily converted to mono-*n*-octylphthalate, its major metabolite, by hydrolysis of a single ester group. Mono-*n*-octylphthalate is detected in the blood of rats within an hour after oral administration of 2,000 mg/kg di-*n*-octylphthalate (Oishi 1990). Hydrolysis of di-*n*-octylphthalate at both ester linkages to produce phthalic acid (minor metabolite) may also occur, but this conversion does not occur readily.

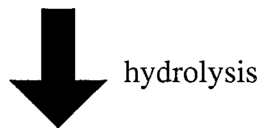
As shown in Figure 2-2, mono-*n*-octylphthalate can undergo ω-, ω-l, α- and β-oxidation to form phthalate monoesters (carboxy, keto, or hydroxy esters), which are the major metabolites detected in the urine (Albro and Moore 1974). Forty-eight hours after the administration of a gavage dose of 559 mg/kg/day of di-*n*-octylphthalate in male CD rats for 2 days, 31% of the administered dose was recovered in the urine as derivatives of the monoester varying in the length of the alkyl side chains (with terminal or subterminal carboxyl, keto, or hydroxyl moieties). The principal urinary metabolite [-$(CH_2)_3COOH$ side chain] resulted from an initial ω-oxidation and two β-oxidations of the *n*-octyl side chain (Albro and Moore 1974). The remaining amount detected in the urine was represented by free phthalic acid and mono-*n*-octylphthalate. The unmetabolized parent compound was not detected.

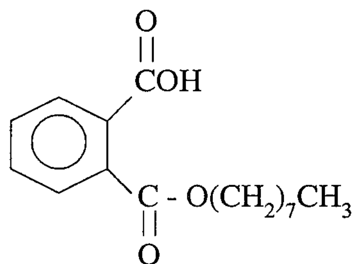
Evidence of the formation of mono-*n*-octylphthalate and phthalate ester metabolites has been shown in *in vitro* studies. The appearance of mono-*n*-octylphthalate was observed with preparations of human small intestine, rat liver and intestine, ferret liver and intestine, and baboon liver and intestine (Lake et al. 1977). However, the amount of phthalic acid and other metabolites in these preparations was either minimal or not detected. The study authors concluded that di-*n*-octylphthalate is probably absorbed primarily as mono-*n*-octylphthalate (Lake et al. 1977). An *in vitro* study reported the formation of five keto acids and two diols when metabolic oxidation of the alkyl groups of di-*n*-octylphthalate was simulated abiotically (Brodsky et al. 1986). Therefore, the *in vivo* and *in vitro* data indicate that major oxidation may occur in the remaining alkyl chain after di-*n*-octylphthalate has been hydrolyzed to the

FIGURE 2-2. Major Metabolic Pathway of Di-*n*-octylphthalate*

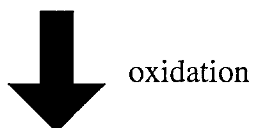


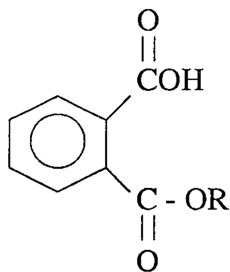
Di-*n*-octylphthalate





Mono-*n*-octylphthalate





R = H (phthalic acid)
= - $(CH_2)_3COOH$
= - $(CH_2)_7COOH$
= - $(CH_2)_6COCH_3$
= - $(CH_2)_6CHOHCH_3$

***Adapted from Albro & Moore 1974**

monoester. It is not known for certain whether di-*n*-octylphthalate is absorbed by the intestine or whether it must first be converted to mono-*n*-octylphthalate.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

No studies were located regarding the excretion of di-*n*-octylphthalate in humans or animals following inhalation exposure.

2.3.4.2 Oral Exposure

No studies were located regarding the excretion of di-*n*-octylphthalate in humans following oral exposure.

Following gavage administration of 559 mg/kg/day of di-*n*-octylphthalate to rats, metabolites accounting for 31% of the administered dose were detected in the urine at 48 hours postexposure (Albro and Moore 1974).

2.3.4.3 Dermal Exposure

No studies were located regarding the excretion of di-*n*-octylphthalate in humans or animals following dermal exposure.

2.4 MECHANISMS OF ACTION

No studies were located regarding mechanisms of action for absorption or distribution of di-n-octylphthalate in humans or animals following inhalation, oral, or dermal exposure.

Di-*n*-octylphthalate has been shown to be a mild liver toxin at high doses in acute- and intermediate-duration studies in rodents. While the mechanism of action for these hepatic effects is not known, din-octylphthalate does not appear to behave like other phthalate esters such as di(2-ethylhexyl)phthalate, which have been shown to be hypolipidemic peroxisome proliferators. Instead, the liver changes

associated with exposure to di-*n*-octylphthalate are characterized by marked centrilobular accumulation of fat and loss of glycogen, accompanied by reduced glucose-6-phosphatase activity and some centrilobular necrosis. However, these effects have been noted in only one study (Mann et al. 1983, and were not seen in the multigeneration study in mice by NTP (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985) or other studies in rats (Lake et al. 1984, 1986; Oishi and Hiraga 1980).

2.5 RELEVANCE TO PUBLIC HEALTH

Populations living in areas surrounding hazardous waste sites may be exposed to di-*n*-octylphthalate primarily via ingestion of drinking water. Other possible routes of exposure are inhalation of contaminated air or dermal contact with contaminated water. For the general population (i.e., including individuals not living in the vicinity of hazardous waste sites), most exposure to di-n-octylphthalate occurs through the use of consumer products containing it. For example, exposure to di-*n*-octylphthalate can occur in people receiving medical treatments that involve the use of polyvinyl chloride tubing from which di-*n*-octylphthalate can leach. Exposure of the general population can also occur by ingestion of contaminated foods into which di-*n*-octylphthalate has leached from packaging materials, by ingestion of contaminated seafood, by drinking contaminated water, or by inhalation of contaminated air. Occupational exposure to di-*n*-octylphthalate can occur in industrial facilities where it is used in the manufacture of plastics or consumer products.

No information is available on the possible health effects of di-*n*-octylphthalate in humans. The liver is the only target organ that has been identified for di-*n*-octylphthalate in animals following acute- and intermediate-duration oral and parenteral exposure. Acute parenteral studies in animals provided data that suggest that di-*n*-octylphthalate may have adverse effects on the immune system, but the relevance of this route of exposure to humans exposed to di-*n*-octylphthalate at hazardous waste sites is not known. Di-*n*-octylphthalate does not appear to induce reproductive toxicity as do other phthalate esters [e.g., di(2-ethylhexyl)phthalate], and oral developmental toxicity studies with di-*n*-octylphthalate have yielded negative results. A decrease in fetal weight and an increase in the incidence of visceral malformations were noted in the offspring of rats administered high doses of di-*n*-octylphthalate by intraperitoneal injection, but the relevance of this study to humans is not known. The only available data on the potential carcinogenicity of di-*n*-octylphthalate suggest that it may be a tumor promoter, but nothing is known about the ability of this compound to induce cancer by itself. *In vitro*

genotoxicity data indicate that di-*n*-octylphthalate is not genotoxic, but there is no information on the *in vivo* genotoxic potential of this compound.

Minimal Risk Levels for Di-n-octylphthalate

Inhalation

No inhalation MRLs were derived for di-*n*-octylphthalate. No data exist on the effects of acute-, intermediate-, or chronic-duration inhalation exposure to di-*n*-octylphthalate.

Oral

Since no human studies were available, animal studies were used for the derivation of the MRL.

- An MRL of 3 mg/kg/day has been derived for acute oral exposure to di-*n*-octylphthalate. This MRL is based on liver effects observed in rats administered di-*n*-octylphthalate via gavage at a dose of 1,000 mg/kg/day (Lake et al. 1986). The hepatic effects consisted of a statistically significant ($p<0.01$) 17% increase in relative liver weight and a statistically significant ($p<0.05$) reduction in enzyme (7-ethoxycoumarin *O*-deethylase) activities. The LOAEL was divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). The choice of liver toxicity as the basis for the acute oral MRL is supported by necrosis and mild hepatic fatty changes seen in other acute- and intermediate-duration studies in rats (DeAngelo et al. 1986; Lake et al. 1984; Mann et al. 1985; Poon et al. 1995).

- An MRL of 0.4 mg/kg/day has been derived for intermediate-duration oral exposure to di-n-octylphthalate. This MRL is based on a NOAEL of 40.8 mg/kg/day for liver effects that were observed in rats fed di-*n*-octylphthalate in the diet at a dose of 350.1 mg/kg/day (males) or 402.9 mg/kg/day (females) (Poon et al. 1995). These hepatic effects consisted of a statistically significant ($p<0.05$) increase in hepatic ethoxyresorufin-0-deethylase activity and histological changes in hepatic architecture, including accentuation of zonation and perivenous cytoplasmic vacuolation. Thyroid toxicity (decreased colloid density and reduced follicle size) was also noted at this concentration. The NOAEL was divided by an uncertainty factor of 100 (10 for

extrapolation from animals to humans and 10 for human variability). Support for the use of hepatic toxicity as the basis of the intermediate MRL is provided by other studies that show necrosis and other fatty changes after acute- and intermediate-duration exposure of rats (DeAngelo et al. 1986; Lake et al. 1984, 1986; Mann et al. 1985).

No chronic oral MRLs were derived for di-*n*-octylphthalate because no reliable data exist on adverse effects of chronic-duration oral exposure to di-*n*-octylphthalate.

Death. No studies were located regarding death in humans after exposure to di-*n*-octylphthalate. LD_{50} values in rodents have been reported for di-*n*-octylphthalate following both oral and parenteral administration. Oral LD_{50} values are reported to be 53,700 mg/kg for rats (Dogra et al. 1987) and 13,000 mg/kg for mice (Dogra et al. 1989). The intraperitoneal LD_{50} in rats is >48,900 mg/kg (Singh et al. 1972). These values indicate that di-*n*-octylphthalate is relatively nonlethal and should not present a risk for death in individuals exposed to this compound in the vicinity of hazardous waste sites.

Systemic Effects

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located regarding gastrointestinal effects in humans following oral exposure to this compound.

No pathological changes of the pancreas were exhibited by rats following intermediate-duration exposure to di-*n*-octylphthalate in the diet (Mann et al. 1985). The available information is insufficient to assess whether adverse gastrointestinal effects are likely to occur in humans exposed to di-*n*-octylphthalate in the vicinity of hazardous waste sites, but the limited information discussed above suggests that such effects are unlikely.

Hematological Effects. No studies were located regarding hematological effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located regarding hematological effects in humans following oral exposure to this compound.

The data from one intermediate-duration oral study in rats suggests that di-*n*-octylphthalate does not cause any hematological effects (Poon et al. 1995), but due to the limited data available, this cannot be stated with certainty.

Hepatic Effects. No studies were located regarding hepatic effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located regarding hepatic effects in humans following oral exposure to this compound.

Results from acute- and intermediate-duration oral studies in small numbers of rats conducted at relatively high doses suggest that the liver is a target for di-*n*-octylphthalate-induced toxicity. Hepatic effects noted in these studies include gross changes in appearance, small but statistically significant increases in relative organ weight, ethoxyresorufin-*O*-deethylase activity, alteration in the activity of several hepatic microsomal enzymes, loss of centrilobular glycogen, cytoplasmic vacuolation, accentuation of zonation, proliferation and dilation of the smooth endoplasmic reticulum accompanied by some loss of rough endoplasmic reticulum, fat accumulation, and occasional necrosis (Lake et al. 1984, 1986; Mann et al. 1985; Oishi and Hiraga 1980, 1982; Poon et al. 1995). In addition, rats exposed to dietary concentrations of mono-*n*-octylphthalate equivalent to 1,000 mg/kg/day exhibited a variety of significant alterations in serum lipid composition, reflecting a possible effect on hepatic metabolism of lipids (Oishi and Hiraga 1982). However, di-*n*-octylphthalate does not appear to behave like other phthalate esters such as di(2-ethylhexyl)phthalate, which has been shown to be a hypolipidemic peroxisome proliferator. Based on these results, adverse hepatic effects may occur in individuals living in the vicinity of hazardous waste sites if di-*n*-octylphthalate is present at sufficiently high levels in the substances consumed (e.g., water).

Renal Effects. No studies were located regarding renal effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located in humans following oral exposure to this compound.

Limited information obtained from oral studies in rats and mice suggests that exposure to di-n-octylphthalate, even at relatively high doses, does not affect the kidney, as evidenced by a lack of change in kidney weight or kidney gross and microscopic pathology (Foster et al. 1980; Heindel et al. 1989; Mann et al. 1985; Morrissey et al. 1989; NTP 1985; Oishi and Hiraga 1980; Poon et al. 1995), although one study noted increased absolute kidney weight in rats with no gross or microscopic

change in the kidney (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985). Therefore, based on this limited information, it does not appear that exposure to di-*n*-octylphthalate at the levels expected to be present in the vicinity of hazardous waste sites is likely to induce adverse renal effects in humans.

Endocrine Effects. No studies were located regarding endocrine effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located in humans following oral exposure to this compound.

Data from acute- and intermediate-duration studies in rats suggests that di-*n*-octylphthalate may cause adverse effects on the thyroid gland. The effects observed include decreased thryoxine levels, histopathological changes (reduced follicle size and colloid density), and ultrastructural changes (enlargement of lysosomes and Golgi apparatus, mitochondrial damage) (Hinton et al. 1986; Poon et al. 1995). Further data are necessary to determine whether thyroid effects might occur in persons living in the vicinity of hazardous waste sites as a result of exposure to di-*n*-octylphthalate.

Dermal Effects. No studies were located regarding dermal effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located in humans following oral exposure to this compound.

In a toxicity summary submitted by Eastman Kodak Company (1978), di-*n*-octylphthalate was reported to be a slight skin irritant when applied to the depilatated skin of guinea pigs, but not a skin sensitizer in guinea pigs. No further details were provided; however, it does not appear that di-*n*-octylphthalate is likely to cause dermal irritation.

Ocular Effects. No studies were located regarding ocular effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located in humans following oral exposure to this compound.

In a toxicity summary submitted by Eastman Kodak Company (1978), ocular administration of di-n-octylphthalate in guinea pigs resulted in slight conjunctival irritation and no cornea1 damage. No further details were provided; however, it does not appear that di-*n*-octylphthalate is likely to cause ocular irritation.

Body Weight Effects. No studies were located regarding body weight effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located in humans following oral exposure to this compound.

Data from various acute- and intermediate-duration oral studies in rats and mice conducted at relatively high doses indicate that exposure to di-*n*-octylphthalate does not adversely affect body weight gain or food consumption (Carter et al. 1992; Heindel et al. 1989; Mann et al 1985; Morrissey et al. 1989; NTP 1985; Oishi and Hiraga 1980, 1982; Poon et al. 1995).

Other Systemic Effects. No studies were located regarding other systemic effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located in humans following oral exposure to this compound.

Although a rough hair coat was observed in mice fed 15,000 mg/kg/day for 14 days (Heindel et al. 1989; NTP 1985), it is not expected that other systemic signs of toxicity would be observed in individuals exposed to di-*n*-octylphthalate in the area surrounding hazardous waste sites.

Immunological and Lymphoreticular Effects. No studies were located regarding immunological or lymphoreticular effects in humans or animals following inhalation exposure to di-*n*-octylphthalate, or in humans following oral or dermal exposure to this compound.

Limited data in rats and mice suggest that di-*n*-octylphthalate can exert immunotoxic effects following acute oral or parenteral exposure to relatively high doses. These effects are reflected in changes in the weight and morphology of various lymphoreticular organs (thymus, spleen, and lymph nodes), altered activity of humoral antibody-forming cells and cellular mediators of immunity, and reduced resistance to bacterial, viral, protozoan, and other parasitic infection (Dogra et al. 1985, 1987, 1989).

The available information suggests that exposure to di-*n*-octylphthalate may adversely affect immune function in individuals living in the vicinity of hazardous waste sites if the individuals ingest sufficiently high levels. Because of its low vapor pressure, exposure to high levels of di-n-octylphthalate by inhalation is not likely.

Neurological Effects. No studies were located regarding neurological effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located regarding neurological effects in humans following oral exposure to di-*n*-octylphthalate.

No clinical signs of neurotoxicity were noted in acute- and intermediate-duration dietary exposure studies using mice (Heindel et al. 1989; NTP 1985). Although these data are limited, it is not believed that the low-level exposure to di-*n*-octylphthalate that occurs at hazardous waste sites will result in neurotoxicity.

Reproductive Effects. No studies were located regarding reproductive effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located in humans following oral exposure to this compound. However, di-*n*-octylphthalate has been shown to cause significant decreases in human sperm motility *in vitro* (Fredricsson et al. 1993).

The results of several acute- and intermediate-duration oral studies in rodents indicate that the potential of di-*n*-octylphthalate to cause adverse reproductive effects is low. Unlike other phthalate esters such as di(2-ethylhexyl)phthalate, di-*n*-octylphthalate does not appear to adversely affect testicular function or morphology (Foster et al. 1980; Gray and Butterworth 1980; Heindel et al. 1989; Morrissey et al. 1989; NTP 1985; Oishi 1990; Oishi and Hiraga 1980; Poon et al. 1995). However, some ultrastructural alterations in Leydig cells, including vesiculation of the smooth endoplasmic reticulum, were noted in rats administered di-*n*-octylphthalate by gavage on 2 consecutive days (Jones et al. 1993). Leydig cells obtained from rats that were cultured and stimulated by LH to measure cellular integrity by examining testosterone output showed decreased testosterone production when incubated with mono-*n*-octylphthalate, the major metabolite of di-*n*-octylphthalate (Jones et al. 1993). Examination of these cells exposed *in vitro* showed that mono-*n*-octylphthalate caused an increase in filopodial proliferation from the cell stroma and basal lamellar processes, dilatation of the smooth endoplasmic reticulum, and mitochondrial swelling and degeneration. No adverse effects on the female estrous cycle or on any index of reproductive function were seen in a multigeneration study in mice (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985). Thus, it is unlikely that individuals exposed to di-*n*-octylphthalate in the vicinity of hazardous waste sites are at risk for adverse reproductive effects resulting from exposure to this compound.

Developmental Effects. No studies were located regarding developmental effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located in humans following oral exposure to this compound.

The results of two oral studies in mice (one being a multigeneration reproductive toxicity study) indicate that di-*n*-octylphthalate has a very low potential to induce adverse developmental effects, especially in view of the very high doses that were evaluated in these studies (Hardin et al. 1987; Heindel et al. 1989; Morrissey et al. 1989; NIOSH 1983; NTP 1985). No statistically significant and/or biologically significant effects were observed with respect to the incidence of skeletal or visceral malformations in offspring exposed *in utero.* A significant decrease in fetal survival was reported in one study (Hardin et al. 1987) of mice given 9,780 mg/kg/day di-*n*-octylphthalate by gavage during gestation days 6-13. A small but significant decrease in average fetal weight and a significantly increased incidence of gross fetal malformations were observed in the offspring of rats administered 4,890 mg/kg/day of di-*n*-octylphthalate by intraperitoneal injection (Singh et al. 1972). Given that the effects seen following parenteral administration may not be relevant to human exposure (e.g., different metabolism), the available information suggests that adverse developmental effects are not likely to occur in humans exposed to di-*n*-octylphthalate in the vicinity of hazardous waste sites.

Genotoxic Effects. No studies were located that assessed the potential, if any, of di-*n*-octylphthalate to induce genotoxic effects in either humans or animals exposed via the inhalation, oral, or dermal routes. No mammalian cell assays on di-*n*-octylphthalate were found.
There is, however, a relatively sizable database of well-conducted microbial assays. As part of the NTP, a series of 34 phthalates or related compounds, including di-*n*-octylphthalate (98%), were evaluated for their potential to induce reverse gene mutations in the *Salmonella typhimurium*/mammalian microsome preincubation assay (Zeiger et al. 1982, 1985). Concentrations of di-*n*-octylphthalate ranging from 100 to 10,000 ug/plate in either the presence or absence of exogenous metabolic activation derived from Aroclor 1254-induced rat or hamster liver fractions were not mutagenic in *S. typhimurium* TA1535, TA1537, TA98, or TAL00. Similar evidence that di-*n*-octylphthalate is not a mutagen for *S. typhimurium* strains has been reported in other preincubation suspension assays (Seed 1982; Shibamoto and Wei 1986) and in plate incorporation assays (Florin et al. 1980; Goodyear 1981a; Sato et al. 1994; Shibamoto and Wei 1986).
Di-*n*-octylphthalate levels ranging from 100 to 2,000 µg/mL (without S9), and 2,000 µg/mL (with

S9, Aroclor 1254-induced rat liver) did not induce deoxyribonucleic acid (DNA) damage in DNA-repair deficient *E. coli* p3478 (Goodyear 1981b). Di-*n*-octylphthalate also showed a negative response in a prokaryotic SOS chromotest assay (Sato et al. 1994). However, the mutagenicity was increased two-fold in the presence of di-*n*-octylphthalate (Sato et al. 1994).

Extracts of waste water, drinking water, soil, or sediment samples collected from various municipal and industrial solid and/or waste water sites were found to be mutagenic in *S. typhimurium* TA98 and TAL00 (Wang et al. 1990). Although di-*n*-octylphthalate (8.9 µg/L) was identified as one the 18 contaminants in the National Bureau of Standards reference sludge sample, several well-characterized mutagens were among the contaminants. It is, therefore, unlikely that the mutagenic activity uncovered in these samples was associated with di-*n*-octylphthalate but rather with the known mutagens that were listed among the 18 contaminants.

Overall, the results of microbial testing indicate that di-*n*-octylphthalate is not a mutagen. Although the database for *in vitro* genetic toxicology testing is limited, the majority of reported studies were well conducted and showed a high degree of concordance. Based on the available information, there is sufficient valid *in vitro* data to conclude that di-*n*-octylphthalate is devoid of genotoxic activity in bacterial test systems. No conclusions can be reached regarding potential effects on other systems *in vitro* or *in vivo*.

Summarized findings from the *in vitro* genotoxicity studies are presented in Table 2-2.

Cancer. No studies were located regarding cancer in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate, and no studies were located in humans following oral exposure to this compound.

Rats exposed to di-*n*-octylphthalate in the diet for either 10 or 26 weeks following a single subcarcinogenic intraperitoneal injection of diethylnitrosamine and partial hepatectomy exhibited increases in GGT-positive liver foci that were not associated with peroxisome proliferation (Carter et al. 1992; DeAngelo et al. 1986). These results suggest that di-*n*-octylphthalate may be effective in promoting preneoplastic lesions in the rat liver, probably by a mechanism that does not rely on peroxisome proliferation.

TABLE 2-2. Genotoxicity of Di-*n*-octylphthalate *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium (TA1535, TA1537, TA98, TA100)	Gene mutation	–[a]	–[a]	Zeiger et al. 1982, 1985
S. typhimurium (TA100)	Gene mutation	–[a]	–[a]	Seed 1982
S. typhimurium (TA98, TA100)	Gene mutation	–[a,b]	–[a,b]	Shibamoto and Wei 1986
S. typhimurium (TA98)	Gene mutation	–[b]	–[b]	Florin et al. 1980
S. typhimurium TA1535, TA1537, TA98, TA100)	Gene mutation	–[b]	–[b]	Goodyear Tire and Rubber Co. 1981a
S. typhimurium (TA98)	Gene mutation	–[b]	–[b]	Sato et al. 1994
Escherichia coli (W3110 [poLA$^+$], p3478 [poLA$_1^-$] DNA)	DNA damage	–[a]	–[a]	Goodyear Tire and Rubber Co. 1981b
E. coli (PQ37)	SOS induction	–	–	Sato et al. 1994

[a]Liquid suspension/preincubation assay
[b]Plate incorporation assay

– = negative result; DNA = deoxyribonucleic acid

The carcinogenic potential of di-*n*-octylphthalate has not been categorized by either IARC, NTP, or EPA.

2.6 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to di-*n*-octylphthalate are discussed in Section 2.6.1.

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*-octylphthalate are discussed in Section 2.6.2.

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 2.8, Populations That Are Unusually Susceptible.

2.6.1 Biomarkers Used to Identify or Quantify Exposure to Di-*n*-octylphthalate

Animal studies have shown that di-*n*-octylphthalate metabolites (primarily the corresponding phthalate monoesters) can be measured in the urine of rats orally exposed to di-*n*-octylphthalate. Therefore, these phthalate monoesters could be useful biomarkers of exposure. There are no other known biomarkers of exposure to di-*n*-octylphthalate.

2.6.2 Biomarkers Used to Characterize Effects Caused by Di-*n*-octylphthalate

No biomarkers of effects caused by di-*n*-octylphthalate have been identified in humans or animals.

2.7 INTERACTIONS WITH OTHER SUBSTANCES

No studies have been identified that investigated the effects of exposure to di-*n*-octylphthalate together with other chemicals. An *in vivo* assay using *S. typhimurium* TA98 showed that di-*n*-octylphthalate enhanced the mutagenicity of two tryptophan pyrolysis products, which is suggestive of increased mutagenic activity in high-temperature cooking if di-*n*-octylphthalate is present (Sato et al. 1994). .

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to di-*n*-octylphthalate than will most persons exposed to the same level of di-*n*-octylphthalate in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters may result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects or clearance rates and any resulting

end-product metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

Studies in animals suggest that, unlike some other phthalate esters, the potential for adverse reproductive or developmental effects following exposure to di-*n*-octylphthalate by the route most relevant to human exposure (oral) is very low (Foster et al. 1980; Gray and Butterworth 1980; Hardin et al. 1987; Heindel et al. 1989; Mann et al. 1985; Morrissey et al. 1989; NIOSH 1983; NTP 1985; Oishi 1990; Oishi and Hiraga 1980). Therefore, it does not appear that individuals of child-bearing age or embryos/fetuses are likely to be unusually susceptible to the effects of di-*n*-octylphthalate. No other information is available on populations with above-average susceptibility to di-*n*-octylphthalate.

2.9 METHODS FOR REDUCING TOXIC EFFECTS

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

2.9.1 Reducing Peak Absorption Following Exposure

Following dermal exposure to di-*n*-octylphthalate, it has been suggested that the skin be washed immediately with copious amounts of soapy water (Stutz and Ulin 1992). If the eyes are exposed to the liquid or vapor, it has been suggested that they be thoroughly flushed with water. Following ingestion of di-*n*-octylphthalate, it has been suggested that one to two glasses of water should be administered (Stutz and Ulin 1992).

2.9.2 Reducing Body Burden

Administration of activated charcoal as an absorptive surface for di-*n*-octylphthalate has been suggested (Stutz and Ulin 1992). If ingestion of large amounts of di-*n*-octylphthalate has occurred, the administration of a cathartic, such as magnesium sulfate, has been shown to increase the elimination of the substance from the gastrointestinal tract (Stutz and Ulin 1992).

2.9.3 Interfering with the Mechanism of Action for Toxic Effects

Di-*n*-octylphthalate has been shown to be a liver toxin at high doses in acute- and intermediate-duration animal studies. Di-*n*-octylphthalate does not appear to behave like other phthalate esters, such as di(2-ethylhexyl)phthalate, which have been shown to be hypolipidemic peroxisome proliferators. Rather, its effects on the liver are more characteristic of other "classic hepatotoxins" (Lake et al. 1984, 1986; Mann et al. 1985). However, the specific mechanism(s) of action for inducing the hepatotoxic effects of di-*n*-octylphthalate is not known. Therefore, there are currently no methods available for interfering with the mechanism of action for the toxic effects of di-*n*-octylphthalate.

2.10 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*-octylphthalate 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*-octylphthalate.

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.

2.10.1 Existing Information on Health Effects of Di-*n*-octylphthalate

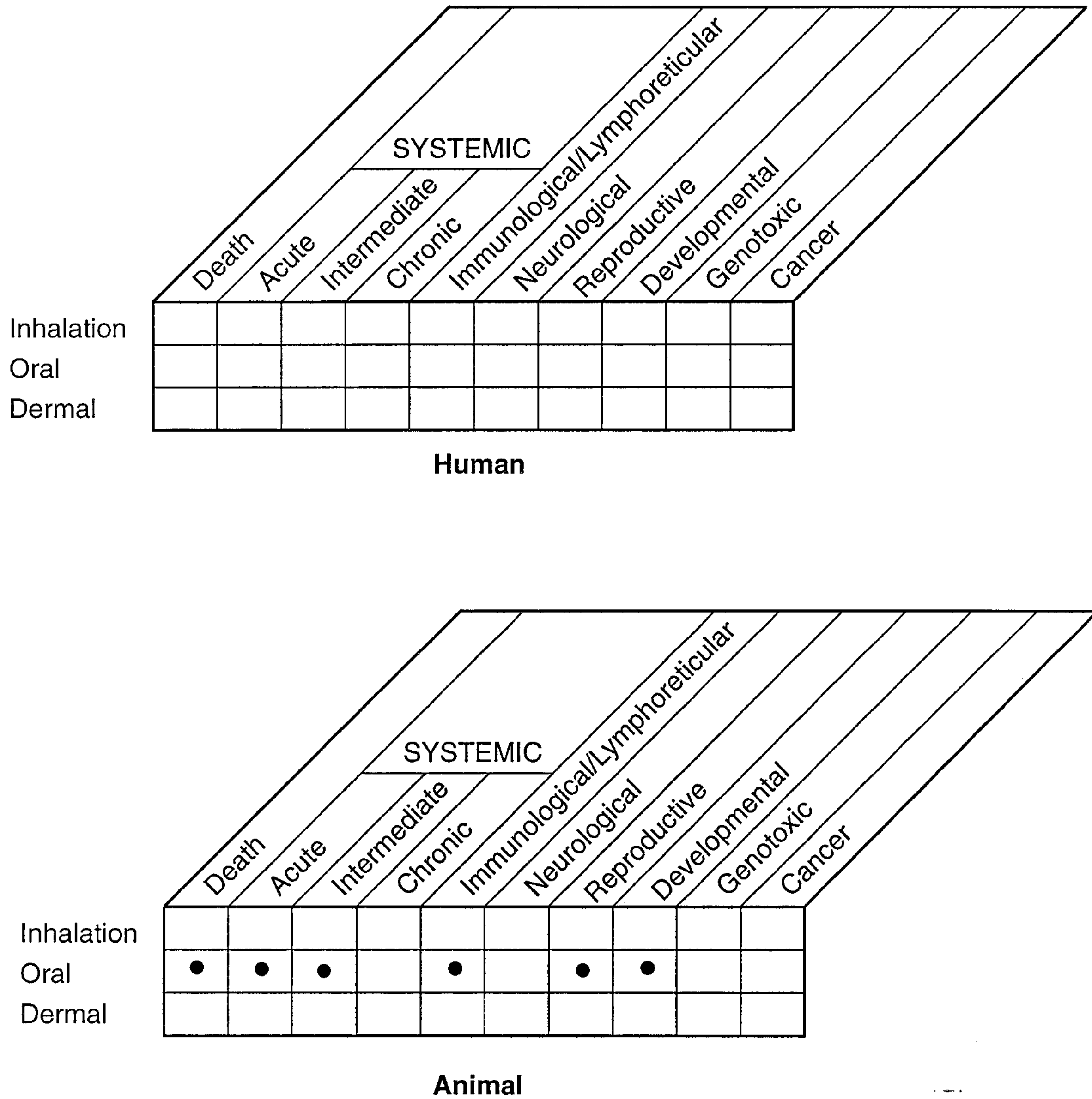
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to di-*n*-octylphthalate are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of di-*n*-octylphthalate. Each dot in the figure 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 1989a), 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.

As can be seen in Figure 2-3, no information is available on the health effects of di-*n*-octylphthalate in humans, and very little information is available in animals. All of the available information on the toxicity of di-*n*-octylphthalate in animals comes from studies in which this compound was administered by either the oral or parenteral route; no information is available from animal studies on the toxicity of di-*n*-octylphthalate following inhalation or dermal exposure. Acute oral and parenteral lethality studies are available in animals, and the hepatic, immunological, reproductive, and developmental toxicity of di-*n*-octylphthalate has been studied following acute- and intermediate-duration parenteral and/or oral exposure in rats and mice. Among reliable studies, the longest duration found for di-*n*-octylphthalate exposure by any route is in a multigeneration reproductive toxicity oral gavage study (85-105 days) in mice and a promotion test dietary study (182 days) in rats.

2.10.2 Identification of Data Needs

Acute-Duration Exposure. There is no information available to identify target organs in humans or animals following acute-duration inhalation or dermal exposure to di-*n*-octylphthalate. No information is available on the effects of acute-duration oral exposure to di-*n*-octylphthalate in humans. Therefore, the data are not sufficient to derive an acute inhalation MRL. An oral LD_{50} of di-*n*-octylphthalate of 53,700 mg/kg has been reported for male rats (Dogra et al. 1987). An oral LD_{50} of 13,000 mg/kg has been reported for mice (Dogra et al. 1989). LD_{50} values are also available for

FIGURE 2-3. Existing Information on Health Effects of Di-*n*-octylphthalate



● Existing Studies

intraperitoneal exposure (Dogra et al. 1985). The liver appears to be the target organ following acute-duration oral exposure to di-*n*-octylphthalate, and an acute oral MRL of 3 mg/kg/day was calculated based on increased relative liver weight and enzyme changes (Lake et al. 1986). Gross and microscopic changes in the liver were observed in rats fed di-*n*-octylphthalate for 10 days (Mann et al. 1985). Decreased thyroxine levels and ultrastructural changes in the thyroid were noted in rats fed di-n-octylphthalate in the diet (2,000 mg/kg/day) for 3 days in the Mann et al. (1985) study (Hinton et al. 1986).

The Mann et al. (1985) study is limited in that too few animals were used, organs other than the liver were not adequately evaluated, and only males were studied. Although an adequate acute-duration oral study would be useful to corroborate or refute the thyroid effects seen in the Mann et al. (1985) study, this does not represent a data need, since an acute oral MRL has been derived. Ingestion of contaminated drinking water is expected to be the predominant route of exposure for individuals living in the vicinity of hazardous waste sites. However, acute-duration inhalation and dermal studies in animals are needed to assess the potential toxicity of di-*n*-octylphthalate following exposure via these routes because there are insufficient pharmacokinetic data available to support the extrapolation of data obtained after oral administration to other routes of exposure.

Intermediate-Duration Exposure. There is no information available to identify target organs in humans or animals following intermediate-duration inhalation or dermal exposure to di-*n*-octylphthalate. Therefore, the data are not sufficient to derive an intermediate-duration inhalation MRL. No information is available on the effects of intermediate-duration oral exposure to di-*n*-octylphthalate in humans. The liver appears to be the target organ following intermediate-duration oral exposure to di-*n*-octylphthalate (DeAngelo et al. 1986; Mann et al. 1985; Poon et al. 1995). An intermediate-duration oral MRL of 0.4 mg/kg/day was calculated based on increases in hepatic ethoxyresorufin-*O*-deethylase activity and histopathological changes in the liver of rats (Poon et al. 1995). Mild microscopic changes were also noted in the thyroid in this study (Poon et al. 1995). Effects on the thyroid (decreased thyroxine levels, reduction in follicle size and colloid density, and ultrastructural changes) have been reported in rats fed diets containing di-*n*-octylphthalate for 21 days in the Mann et al. (1985) study (Hinton et al. 1986) or 13 weeks (Poon et al. 1995). Both the Mann et al. (1985) and the DeAngelo et al. (1986) studies are limited in that too few animals were used, organs other than the liver were not adequately evaluated, and only males were studied. Because statistical analysis was not performed on the data in the Poon et al. (1995) study and the thyroid

effects that were observed were mild, it is difficult to determine at which concentration the LOAEL for these particular effects occurred. Although ingestion of contaminated drinking water is expected to be the predominant route of exposure for individuals living in the vicinity of hazardous waste sites, intermediate-duration inhalation and dermal studies in animals are needed to assess the potential toxicity of di-*n*-octylphthalate following these routes of exposure because there are insufficient pharmacokinetic data available to support the extrapolation of data obtained after oral administration to other routes of exposure.

Chronic-Duration Exposure and Cancer. There is no information available to identify target organs in humans following chronic-duration inhalation, oral, or dermal exposure to di-*n*-octylphthalate. Therefore, the data are not sufficient to derive a chronic-duration inhalation MRL.
Chronic-duration oral toxicity studies using di-*n*-octylphthalate are needed to identify target organs and to establish the levels at which effects may occur. Oral studies are needed because ingestion of contaminated drinking water is expected to be the predominant route of exposure for individuals living in the vicinity of hazardous waste sites.

No studies were located regarding cancer in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate, and no studies were located in humans following oral exposure to this compound. Rats exposed to di-*n*-octylphthalate in the diet for either 10 or 26 weeks following a single subcarcinogenic intraperitoneal injection of diethylnitrosamine and partial hepatectomy exhibited increases in GGT-positive liver foci that were not associated with a peroxisome proliferation (Carter et al. 1992; DeAngelo et al. 1986). These results suggest that di-*n*-octylphthalate may be effective in promoting preneoplastic lesions in the rat liver, probably by a mechanism that does not rely on peroxisome proliferation. An oral cancer bioassay would be useful to establish whether di-n-octylphthalate has the potential to be carcinogenic to humans.

Genotoxicity. There is convincing evidence from microbial assays that di-*n*-octylphthalate is not a mutagen in *S. typhimurium* (Florin et al. 1980; Goodyear 1981a; Sato et al. 1994; Seed 1982; Shibamoto and Wei 1986; Zeiger et al. 1982, 1985) and does not induce DNA damage in *E. coli* (Goodyear 1981b). Although genetic toxicology testing, particularly in mammalian cell systems, is limited, the reported studies were well conducted and uniformly negative. It is, therefore, doubtful whether further investigation of these end points in other mammalian cell lines would alter the negative conclusions. Of greater importance, however, is the demonstrated lack of mutagenesis of the

rodent hepatocarcinogen, di(2-ethylhexyl)phthalate in a similar battery of *in vitro* tests. The inactivity of this carcinogenic phthalate suggests that *in vitro* genetic toxicology assays may have limited value for predicting the carcinogenic potential of other phthalates such as di-*n*-octylphthalate. Nevertheless, data from whole-animal studies using di-*n*-octylphthalate are needed since no literature exists on potential adverse genetic effects *in vivo.*

Reproductive Toxicity. No studies were located regarding reproductive effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate. No studies were located in humans following oral exposure to this compound. Di-*n*-octylphthalate caused significant decreases in human sperm motility *in vitro* (Fredricsson et al. 1993). The results of several acute- and intermediate-duration oral studies in rodents indicate that the potential of di-*n*-octylphthalate to cause adverse reproductive effects is low. Unlike other phthalate esters such as di(2-ethylhexyl)phthalate, di-n-octylphthalate does not appear to adversely affect testicular function or morphology (Foster et al. 1980; Gray and Butterworth 1980; Heindel et al. 1989; Morrissey et al. 1989; NTP 1985; Oishi 1990; Oishi and Hiraga 1980; Poon et al. 1995). However, some ultrastructural alterations in Leydig cells, including vesiculation of the smooth endoplasmic reticulum, were noted in rats administered di-*n*-octylphthalate by gavage on 2 consecutive days (Jones et al. 1993). Leydig cells obtained from rats that were cultured and stimulated by LH to measure cellular integrity by examining testosterone output showed decreased testosterone production when incubated with mono-*n*-octylphthalate, the major metabolite of di-*n*-octylphthalate (Jones et al. 1993). Examination of these cells exposed *in vitro* showed that mono-*n*-octylphthalate caused ultrastructural changes in several organelles, including the smooth endoplasmic reticulum dilatation and mitochondrial degeneration. No adverse effects on the female estrous cycle or on any index of reproductive function were seen in a multigeneration study in mice (Heindel et al. 1989; Morrissey et al. 1989; NTP 1985). Although it is fairly well established that di-*n*-octylphthalate does not induce adverse effects on male reproductive organs or reproductive performance in either males or females, data on reproductive organ pathology, including ultra-structural pathology, are needed in any 90-day studies that may be conducted with di-*n*-octylphthalate.

Developmental Toxicity. No studies were located regarding developmental effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate, and no studies were located in humans following oral exposure to this compound. The results of two oral studies in mice (one being a multigeneration reproductive toxicity study) indicate that di-*n*-octylphthalate has a very low potential to induce adverse developmental effects, especially in view of the very high doses that were evaluated

in these studies (Hardin et al. 1987; Heindel et al. 1989; Morrissey et al. 1989; NIOSH 1983; NTP 1985). No statistically significant and/or biologically significant effects were observed with respect either to embryo or fetal survival or growth, or to the incidence of skeletal or visceral malformations in offspring exposed *in utero.* However, a significant decrease in average fetal weight and a significantly increased incidence in gross fetal malformations were observed in the offspring of rats administered 4,890 mg/kg/day of di-*n*-octylphthalate by intraperitoneal injection (Singh et al. 1972). However, the effects seen following high-dose parenteral administration may not be relevant to human exposure. Well-conducted oral developmental toxicity studies in animals are needed to determine whether the negative results obtained in the two studies discussed above (one being a screen and the other being a multigeneration reproductive toxicity study not designed specifically to assess developmental toxicity) are valid, or if the effects seen after intraperitoneal administration of high doses of di-*n*-octylphthalate are likely to occur after oral administration. There are insufficient pharmacokinetic data available to support the extrapolation of data obtained after oral administration to other routes of exposure. However, oral studies would be the most useful since ingestion of contaminated drinking water is expected to be the predominant route of exposure for individuals living in the vicinity of hazardous waste sites.

Immunotoxicity. No studies were located regarding immunological effects in humans or animals following inhalation or dermal exposure to di-*n*-octylphthalate, or in humans following oral exposure to this compound. Limited data in rats or mice suggest that di-*n*-octylphthalate can exert immunotoxic effects following acute oral or parenteral exposure to relative high doses. These effects are reflected in changes in the weight and morphology of various lymphoreticular organs (thymus, spleen, and lymph nodes), altered activity of humoral antibody-forming cells and cellular mediators of immunity, and reduced resistance to bacterial, viral, protozoan, or other parasitic infection (Dogra et al. 1985, 1987, 1989). Additional data are needed to measure lymphoreticular organs and blood components of the immune system in any 90-day study that may be conducted with di-*n*-octylphthalate because the limited information available from animal studies suggests that this compound may exert immunotoxic effects.

Neurotoxicity. No information is available on the neurological effects of di-*n*-octylphthalate in humans or animals following inhalation or dermal exposure or in humans following oral exposure. No clinical signs of neurotoxicity were noted in acute and intermediate duration dietary exposure studies using mice (Heindel et al. 1989; NTP 1985). Although these data are limited, it is not believed that

the low-level exposure to di-*n*-octylphthalate that occurs at hazardous waste sites will result in neurotoxicity. Because there is no information to suggest that the central nervous system is a target of di-*n*-octylphthalate, no additional information is needed at this time.

Epidemiological and Human Dosimetry Studies. No epidemiological studies are available on populations that have been exposed solely to di-*n*-octylphthalate. As a result of its use, together with other phthalate esters, as a plasticizer in the production of polyvinyl chloride (PVC) resins and cellulose ester and polystyrene resins (EPA 1993a; HSDB 1995; Mannsville Chemical Products Corporation 1989) exposure of the general population and of workers in occupational settings is significant. Therefore, it is unlikely that both a specific subpopulation exposed only to di-*n*-octylphthalate and a control population with no known exposure could be identified. However, if suitable subpopulations could be found, then a well-conducted and controlled epidemiological study is needed to determine the potential target organs of di-*n*-octylphthalate toxicity in humans and the levels at which effects might be expected to occur. In addition, individuals at risk in the vicinity of hazardous waste sites could be identified and monitored.

Biomarkers of Exposure and Effect

Exposure. The monoester derivatives of di-*n*-octylphthalate and mono-*n*-octylphthalate or the oxidation products of mono-*n*-otylphthalates could potentially be used as a biomarker of exposure; however, only a few studies have been located that measure these metabolites in body tissues or fluids following exposure to di-*n*-octylphthalate (Albro and Moore 1974; Oishi 1990). Studies that investigate the fate and/or elimination of these metabolites are needed to determine its value as a biomarker of exposure for di-*n*-octylphthalate. Additional information on the metabolism of di-*n*-octylphthalate could help identify other potential biomarkers of exposure.

Effect. Since exposure to di-*n*-octylphthalate does not produce a unique clinical disease state, no biomarkers of effect have been identified. Additional information on the potential health effects of di-*n*-octylphthalate is needed to identify biomarkers of exposure to this compound.

Absorption, Distribution, Metabolism, and Excretion. No studies were located regarding the absorption of di-*n*-octylphthalate in humans and animals following inhalation and dermal exposure. Information on absorption in humans following oral exposure is not available. There are studies that suggest oral absorption of di-*n*-octylphthalate occurs in animals (Albro and Moore 1974; Oishi 1990; Poon et al. 1995); however, quantitative information is lacking. Additional information, primarily quantitative data, on absorption of di-*n*-octylphthalate for all routes of exposure is needed to understand and predict effects.

Information on the distribution of di-*n*-octylphthalate is limited to oral studies in rats, one by Oishi (1990), which reported the identification of mono-*n*-octylphthalate in blood and testes with peak levels observed at 3 hours for blood and at 6 hours for testes after dosing, and the other by Poon et al. (1995), which reported di-*n*-octylphthalate in the liver that was either below or slightly above detection limits; higher levels (15-25 ppm) of residue were also found in adipose tissue. However, this latter study was limited because metabolite levels were not measured. The metabolism of di-*n*-octylphthalate following acute exposure has been studied in animals *in vivo* and *in vitro* (Albro and Moore 1974; Brodsky et al. 1986; Lake et al. 1977). Metabolism studies following longer term exposures are needed in order to determine if metabolic pathways become saturated or altered. Although the Albro and Moore (1974) study seems to indicate that urine is the major elimination route of di-*n*-octylphthalate, additional excretion studies are needed to provide quantitative information. Additional studies on the mechanism involved in absorption and distribution of the compound are needed to provide information on how to increase elimination of the compound from the body.

Comparative Toxicokinetics. Based on the rat study by Albro and Moore (1974), di-*n*-octylphthalate appears to be readily absorbed following oral administration, metabolized extensively, and excreted primarily in the urine. Because of the lack of human data and limited animal data on the absorption, distribution, metabolism, and excretion of di-*n*-octylphthalate, additional studies are needed in order to make comparisons on the toxicokinetics across species.

Methods for Reducing Toxic Effects. All of the treatment methods currently available for use in di-*n*-octylphthalate ingestion or skin contact are supportive in nature and/or involve decreasing the absorption or increasing the rate of elimination of di-*n*-octylphthalate (Stutz and Ulin 1992). Since the mechanism of di-*n*-octylphthalate toxicity is not known, there are currently no methods that focus on mitigating the effects of di-*n*-octylphthalate by interfering with its mode of action. Therefore, more

information on the mechanism of action for di-*n*-octylphthalate is needed in order to devise methods for the mitigation of its toxic effects.

2.10.3 On-going Studies

No on-going studies on the health effects or toxicokinetics of di-*n*-octylphthalate were found.