3. HEALTH EFFECTS

3.1 INTRODUCTION

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

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

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

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

considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of dichlorobenzenes are indicated in Tables 3-1 and 3-5 and Figures 3-1 and 3-5.

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.

3.2.1 Inhalation Exposure

Descriptive data are available from reports of humans exposed to 1,2- and 1,4-DCB by inhalation (and possibly dermal contact). It is important to note that the case studies discussed in this section should be interpreted with caution since they reflect incidents in which individuals have reportedly been exposed to 1,2- and 1,4-DCB, and they assume that there has been no other exposure to potentially toxic or infectious agents. There is usually little or no verification of these assumptions, and often no estimate of the level of exposure which may have occurred. With only rare exceptions, case studies in general are not scientifically equivalent to carefully designed epidemiological studies or to adequately controlled and monitored laboratory experiments. Thus, the case studies described below should be considered only as providing supplementary evidence that 1,2- and 1,4-DCB may cause the reported human effects. The highest NOAEL and all reliable LOAEL values after inhalation exposure to 1,2- and 1,4-DCB are recorded in Tables 3-1 and 3-2, respectively, and plotted in Figures 3-1 and 3-2, respectively. No LSE tables or figures were generated for 1,3-DCB due to a lack of inhalation data.

Table 3-1 Levels of Significant Exposure to 1,2-dichlorobenzene - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	6 hr				1532 M (14-day LC50)	Bonnet et al. 1982 1,2-dichlorobenzene	
2	Mouse (Sprague-Dawley)	6 hr				1236 (14-day LC50)	Bonnet et al. 1982 1,2-dichlorobenzene	
Systemic								
3	Rat (Sprague-Dawley)	4 hr	Hemato	29 M			Brondeau et al. 1990 1,2-dichlorobenzene	
4	Rat (NS)	10 d 6 hr/d	Hepatic	322			DuPont 1982 1,2-dichlorobenzene	
			Renal	322				
			Bd Wt		322 (slight body weight loss)			
5	Rat (Fischer- 344)	10 d Gd 6-18 6 hr/d	Bd Wt		100 F (reduced maternal body weight gain throughout gestation)		Hayes et al. 1985 1,2-dichlorobenzene	
6	Rat (albino)	0.5 hr (NS)	Hepatic		977 M (marked central lobular necrosis)		Hollingsworth et al. 1958 1,2-dichlorobenzene	
			Renal		977 M (cloudy swelling of tubular epithelium)			

Table 3-1 Levels of Significant Exposure to 1,2-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
7	Rat (albino)	1 hr (NS)	Hepatic		977 M (marked central lobular necrosis)		Hollingsworth et al. 1958 1,2-dichlorobenzene	
			Renal		977 M (cloudy swelling of tubular epithelium)			
8	Rat (albino)	3 hr (NS)	Hepatic	539 M			Hollingsworth et al. 1958 1,2-dichlorobenzene	
			Renal	539 M				
9	Rat (albino)	6.5 hr (NS)	Hepatic	539 M			Hollingsworth et al. 1958 1,2-dichlorobenzene	
			Renal	539 M				
10	Mouse	4-14 d 5 d/wk 6 hr/d (NS)	Resp			64 M (moderate to severe nasal olfactory epithelial lesions)	Zissu 1995 1,2-dichlorobenzene	
11	Rabbit (New Zealand)	13 d Gd 6-18 6 hr/d	Bd Wt		100 F (slight maternal body weight loss on Gd 6-8 followed by recovery)		Hayes et al. 1985 1,2-dichlorobenzene	
Reproductive								
12	Rat (Fischer- 344)	10 d Gd 6-18 6 hr/d		400 F			Hayes et al. 1985 1,2-dichlorobenzene	

Table 3-1 Levels of Significant Exposure to 1,2-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
13	Rabbit (New Zealand)	13 d Gd 6-18 6 hr/d		400 F			Hayes et al. 1985 1,2-dichlorobenzene	
Developmental								
14	Rat (Fischer- 344)	10 d Gd 6-18 6 hr/d		200 F	400 F (delayed ossification of cervical vertebral centra)		Hayes et al. 1985 1,2-dichlorobenzene	
15	Rabbit (New Zealand)	13 d Gd 6-18 6 hr/d		400 F			Hayes et al. 1985 1,2-dichlorobenzene	
INTERMEDIATE EXPOSURE								
Systemic								
16	Rat (CD)	2 generations 7 hr/d 6 d/wk	Hepatic	50	150 (centrilobular hepatocellular hypertrophy in F0 and F1 adults)		Bio/dynamics 1989 1,2-dichlorobenzene	
			Bd Wt	50	150 (reduced body weight gain in F0 and F1 adults)			

Table 3-1 Levels of Significant Exposure to 1,2-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
17	Rat (albino)	6-7 mo 5 d/wk 7 hr/d (NS)	Resp	93 M			Hollingsworth et al. 1958 1,2-dichlorobenzene	
			Cardio	93 M				
			Hepatic	93 M				
			Renal	93 M				
			Bd Wt	49 M	93 M (9.3% reduced body weight gain)			
18	Mouse (NS)	6.5 mo 5 d/wk 7 hr/d (NS)	Resp	49 F			Hollingsworth et al. 1958 1,2-dichlorobenzene	
			Hepatic	49 F				
			Renal	49 F				
			Bd Wt	49 F				
19	Gn Pig (NS)	6-7 mo 5 d/wk 7 hr/d (NS)	Resp	93			Hollingsworth et al. 1958 1,2-dichlorobenzene	
			Cardio	93				
			Hepatic	93				
			Renal	93				
			Bd Wt	93				

Table 3-1 Levels of Significant Exposure to 1,2-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
20	Rabbit (albino)	6-7 mo 5 d/wk 7 hr/d (NS)	Resp	93			Hollingsworth et al. 1958 1,2-dichlorobenzene	
			Cardio	93				
			Hemato	93				
			Hepatic	93				
			Renal	93				
			Bd Wt	93				
Immuno/ Lymphoret								
21	Rat (albino)	6-7 mo 5 d/wk 7 hr/d (NS)		93			Hollingsworth et al. 1958 1,2-dichlorobenzene	
22	Gn Pig (NS)	6-7 mo 5 d/wk 7 hr/d (NS)			93 M (20% reduced absolute spleen weight)		Hollingsworth et al. 1958 1,2-dichlorobenzene	
Reproductive								
23	Rat (CD)	2 generations 7 hr/d 6 d/wk		394			Bio/dynamics 1989 1,2-dichlorobenzene	
24	Rat (albino)	6-7 mo 5 d/wk 7 hr/d (NS)		93 M			Hollingsworth et al. 1958 1,2-dichlorobenzene	

Table 3-1 Levels of Significant Exposure to 1,2-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
25	Gn Pig (albino)	6-7 mo 5 d/wk 7 hr/d (NS)		93 M			Hollingsworth et al. 1958 1,2-dichlorobenzene	

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

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); F = Female; Gd = gestational day; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LC50 = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; ppm = parts per million; Resp = respiratory; wk = week(s)

Figure 3-1 Levels of Significant Exposure to 1,2-dichlorobenzene - Inhalation

Acute (≤14 days)

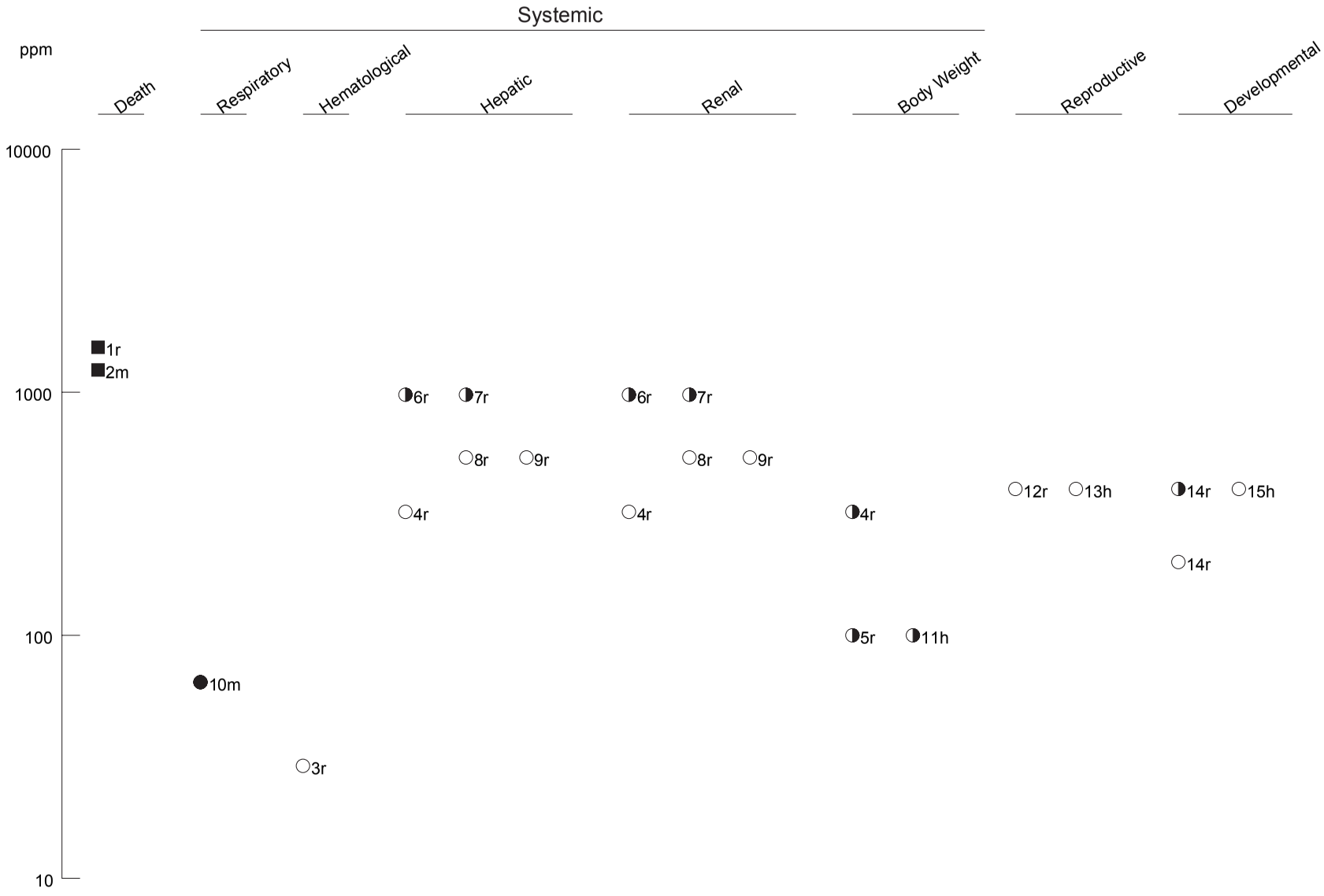


Figure 3-1 Levels of Significant Exposure to 1,2-dichlorobenzene - Inhalation *(Continued)*

Intermediate (15-364 days)

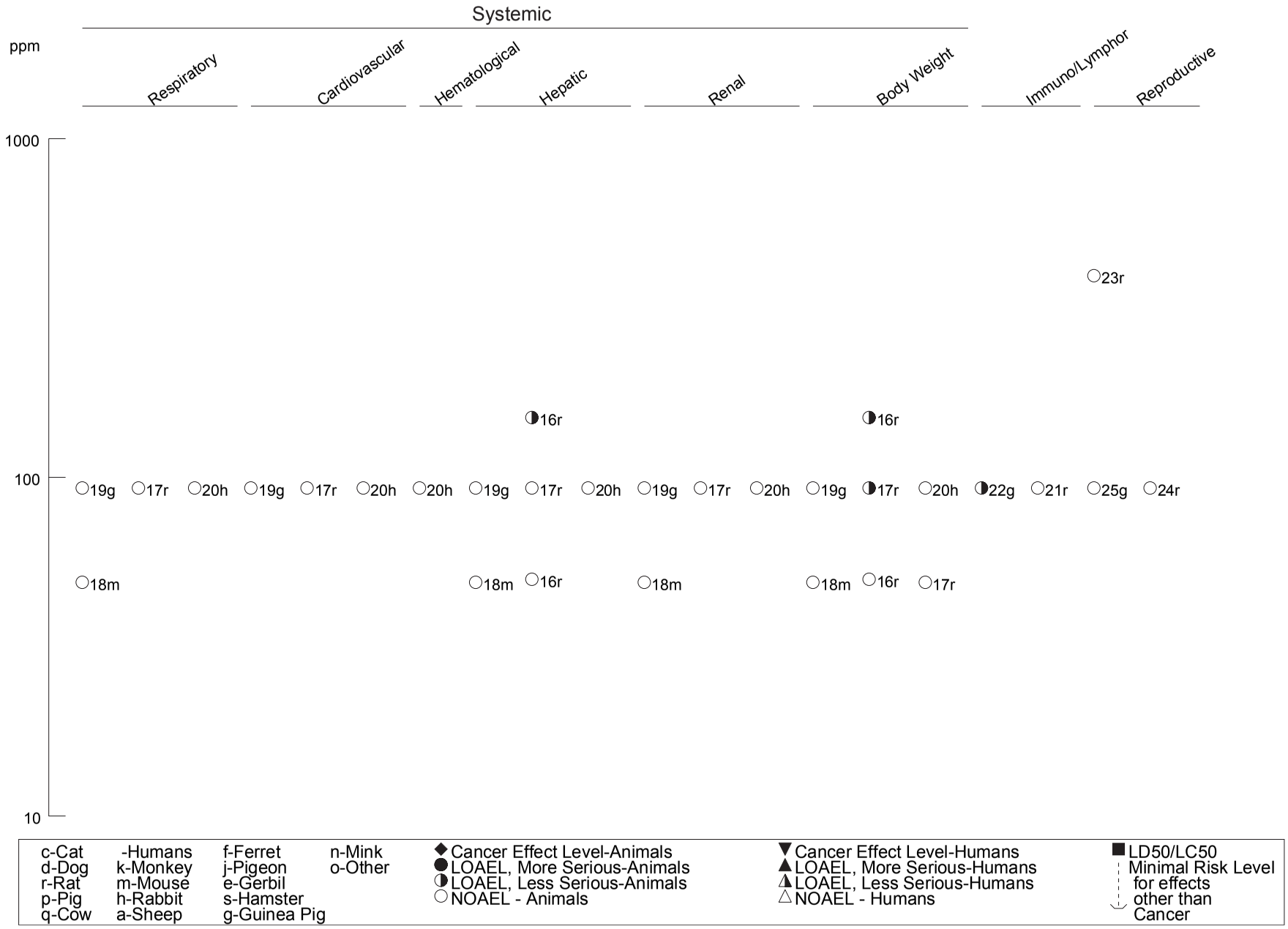


Table 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Systemic								
1	Human	4.75 yr 5 d/wk 8 hr/d (occup)	Resp	15 M[b]	30 M (minimal nose and eye irritation)	50 M (painful irritation of nose and eyes)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
2	Rat (Alderley-Park)	10 d Gd 6-15 6 hr/d	Resp	508 F			Hodge et al. 1977 1,4-dichlorobenzene	
			Cardio	508 F				
			Hepatic	508 F				
			Renal	508 F				
			Bd Wt	508 F				
3	Rabbit (New Zealand)	13 d Gd 6-18 6 hr/d	Bd Wt	300 F	800 F (slight maternal body weight loss on Gd 6-8 followed by recovery)		Hayes et al. 1985 1,4-dichlorobenzene	
Reproductive								
4	Rat (Alderley-Park)	10 d Gd 6-15 6 hr/d		500 F			Hodge et al. 1977 1,4-dichlorobenzene	
5	Rabbit (New Zealand)	13 d Gd 6-18 6 hr/d		800 F			Hayes et al. 1985 1,4-dichlorobenzene	
Developmental								
6	Rat (Alderley-Park)	10 d Gd 6-15 6 hr/d		508 F			Hodge et al. 1977 1,4-dichlorobenzene	

Table 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
7	Rabbit (New Zealand)	13 d Gd 6-18 6 hr/d		300 F	800 F (increased incidence of retroesophageal right subclavian artery)		Hayes et al. 1985 1,4-dichlorobenzene	
INTERMEDIATE EXPOSURE								
Death								
8	Rat (NS)	9-12 wk 5 d/wk 8 hr/d				798 (2/19 males and 2/15 females died)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
9	Gn Pig (NS)	4-4.5 wk 5 d/wk 8 hr/d				798 M (2/16 died)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
10	Rabbit (NS)	12 wk 5 d/wk 8 hr/d				798 (3 males and 1 female died)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
Systemic								
11	Rat (Fischer- 344)	13 wk 5 d/wk 6 hr/d	Resp	600			Aiso et al. 2005a 1,4-dichlorobenzene	
			Hepatic	120 M	270 M (increased liver weight, serum cholesterol, and serum phospholipids)			
			Bd Wt	600				

Table 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
12	Rat (NS)	2-12 wk 5 d/wk 7 or 8 hr/d	Resp	798 F		173 M (slight interstitial edema, alveolar hemorrhage)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
			Cardio	173				
			Hepatic		173 F (slight liver congestion and granular degeneration)	798 (cloudy swelling and central necrosis)		
			Renal		173 (increased relative kidney weight)			
			Ocular		798 (eye irritation)			
			Bd Wt	173	798 (unquantitated weight loss)			
13	Rat (NS)	5.1-7.1 mo 5 d/wk 7 hr/d	Hemato	96			Hollingsworth et al. 1956 1,4-dichlorobenzene	
			Hepatic	96	158 (increased relative liver weight; cloudy swelling or degeneration of parenchyma)			
			Renal	96	158 M (increased relative kidney weight)			
			Bd Wt	341				

Table 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
14	Rat (Sprague-Dawley)	2 generations	Resp	211	538		Tyl and Neeper-Bradley 1989 1,4-dichlorobenzene	
			Hepatic	66[c] M	211[d] M (increased liver weight)			
				211 F	538 F			
			Renal	538 F				
			Ocular	211	538 (encrustation of periocular region; lacrimation)			
			Bd Wt	66[d] M	211[d] M (decreased body weight in the male F0 group and in the F1 male and females in the 5-week recovery study)			
				211 F				
					538 F			
			Other	211	538 (decreased grooming; unkempt appearance; decreased food consumption)			

Table 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
15	Mouse BDF1	13 wk 5 d/wk 6 hr/d	Resp	600			Aiso et al. 2005a 1,4-dichlorobenzene	
			Hemato	600				
			Hepatic	120 M	270 M (increased relative liver weight and serum ALT)			
			Renal	600				
			Bd Wt	600				
16	Mouse (NS)	5.1-7.1 mo 5 d/wk 7 hr/d	Hepatic	158 M 96[d] F			Hollingsworth et al. 1956 1,4-dichlorobenzene	
			Renal	158 M 96[d] F				
			Bd Wt	158 M 96[d] F				
17	Gn Pig (NS)	5.1-7.1 mo 5 d/wk 7 hr/d	Hepatic	96	158 F (increased relative liver weight)	341 (focal necrosis, slight cirrhosis in males)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
			Renal	341				
			Bd Wt	96	158 (slight depression in final body weight)			

Table 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form	Comments
18	Gn Pig (NS)	2-4.5 wk 5 d/wk 7 or 8 hr/d	Resp		173 F	(alveolar hemorrhage and edema)			Hollingsworth et al. 1956 1,4-dichlorobenzene	
			Cardio	798						
			Hepatic	173			798	(cloudy swelling in the liver and central necrosis)		
			Renal	798						
			Ocular	173	798	(eye irritation)				
			Bd Wt	173	798	(body weight loss, not quantified)				
19	Rabbit (NS)	2-12 wk 5 d/wk 7 or 8 hr/d	Resp		173 F	(lung congestion and interstitial edema)	798	(emphysema in 2/8)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
			Hepatic	173			798	(cloudy swelling in the liver and central necrosis)		
			Renal	798						
			Ocular		798	(eye irritation; reversible nonspecific eye changes)				
			Bd Wt	173	798	(decreased body weight gain, not quantitated)				

Table 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)		Reference Chemical Form	Comments
Immuno/ Lymphoret									
20	Gn Pig (Hartley)	12 wk		50 M				Suzuki et al. 1991 1,4-dichlorobenzene	
Neurological									
21	Rat (NS)	9-12 wk 5 d/wk 8 hr/d				798	(tremors, weakness, unconsciousness)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
22	Rat	2 generations		211		538	(tremors and other signs of neurotoxity in F0 and F1 adults)	Tyl and Neeper-Bradley 1989 1,4-dichlorobenzene	
23	Gn Pig (NS)	4-4.5 wk 5 d/wk 8 hr/d				798	(tremors, weakness, unconsciousness)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
24	Rabbit (NS)	12 wk 5 d/wk 8 hr/d				798	(tremors, weakness, unconsciousness)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
Reproductive									
25	Rat (NS)	5.1-7.1 mo 5 d/wk 7 hr/d		158 M				Hollingsworth et al. 1956 1,4-dichlorobenzene	
26	Rat (NS)	16 d 5 d/wk 7 hr/d		173 M				Hollingsworth et al. 1956 1,4-dichlorobenzene	
27	Rat (Sprague-Dawley)	2 generations		538				Tyl and Neeper-Bradley 1989 1,4-dichlorobenzene	

Table 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
28	Gn Pig (NS)	16 d 5 d/wk 7 hr/d		173 M			Hollingsworth et al. 1956 1,4-dichlorobenzene	
29	Gn Pig (NS)	5.1-7.1 mo 5 d/wk 7 hr/d		158 M			Hollingsworth et al. 1956 1,4-dichlorobenzene	
Developmental								
30	Rat (Sprague-Dawley)	2 generations		93 211		538 (increased stillbirths and reduced postnatal survival in F1 and F2 pups)	Tyl and Neeper-Bradley 1989 1,4-dichlorobenzene	
CHRONIC EXPOSURE								
Death								
31	Rat (Fischer- 344)	104 wk 5 d/wk 6 hr/d chamber				300 M (30% reduced survival)	Aiso et al. 2005b 1,4-dichlorobenzene	

Table 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
Systemic								
32	Rat (Fischer- 344)	104 wk 5 d/wk 6 hr/d chamber	Resp	75 M 20[e] F	300 M (eosinophilic changes in olfactory epithelium) 75[d] F (eosinophilic changes in nasal olfactory epithelium)		Aiso et al. 2005b 1,4-dichlorobenzene	
			Renal	75 M	300 M (mineralization of renal papilla, urothelial hyperplasia)			
			Bd Wt	300				
33	Mouse Crj:BDF1	104 wk 5 d/wk 6 hr/d chamber	Resp	75 F	300 F (metaplasia in nasal olfactory epithelium)		Aiso et al. 2005b 1,4-dichlorobenzene	
			Hepatic	75[d] M 300 F	300 M (centrilobular hepatocellular hypertrophy)			
			Bd Wt	75	300 (reduced terminal body weight)			
Reproductive								
34	Rat (Fischer- 344)	104 wk 5 d/wk 6 hr/d chamber		300			Aiso et al. 2005b 1,4-dichlorobenzene	

Table 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
35	Mouse Crj:BDF1	104 wk 5 d/wk 6 hr/d chamber		300			Aiso et al. 2005b 1,4-dichlorobenzene	
Cancer								
36	Mouse Crj:BDF1	104 wk 5 d/wk 6 hr/d chamber				300 M (CEL: hepatocellular carcinoma, hepatic histiocytic sarcoma)	Aiso et al. 2005b 1,4-dichlorobenzene	
						300 F (CEL: bronchoalveolar adenoma and carcinoma)		
						10 F (CEL: hepatocellular adenoma and carcinoma)		

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

b Used to derive an acute-duration inhalation minimal risk level (MRL) of 2 ppm. The MRL was obtained by dividing the NOAEL by an uncertainty factor of 10 (for human variability).

c Study result used to derive an intermediate-duration inhalation Minimal Risk Level (MRL) of 0.2 ppm for 1,4-DCB, as described in detail in Appendix A. Benchmark dose analysis was performed on liver weight to select a point of departure, which was adjusted for intermittent exposure and converted to a Human Equivalent Concentration (HEC), then divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

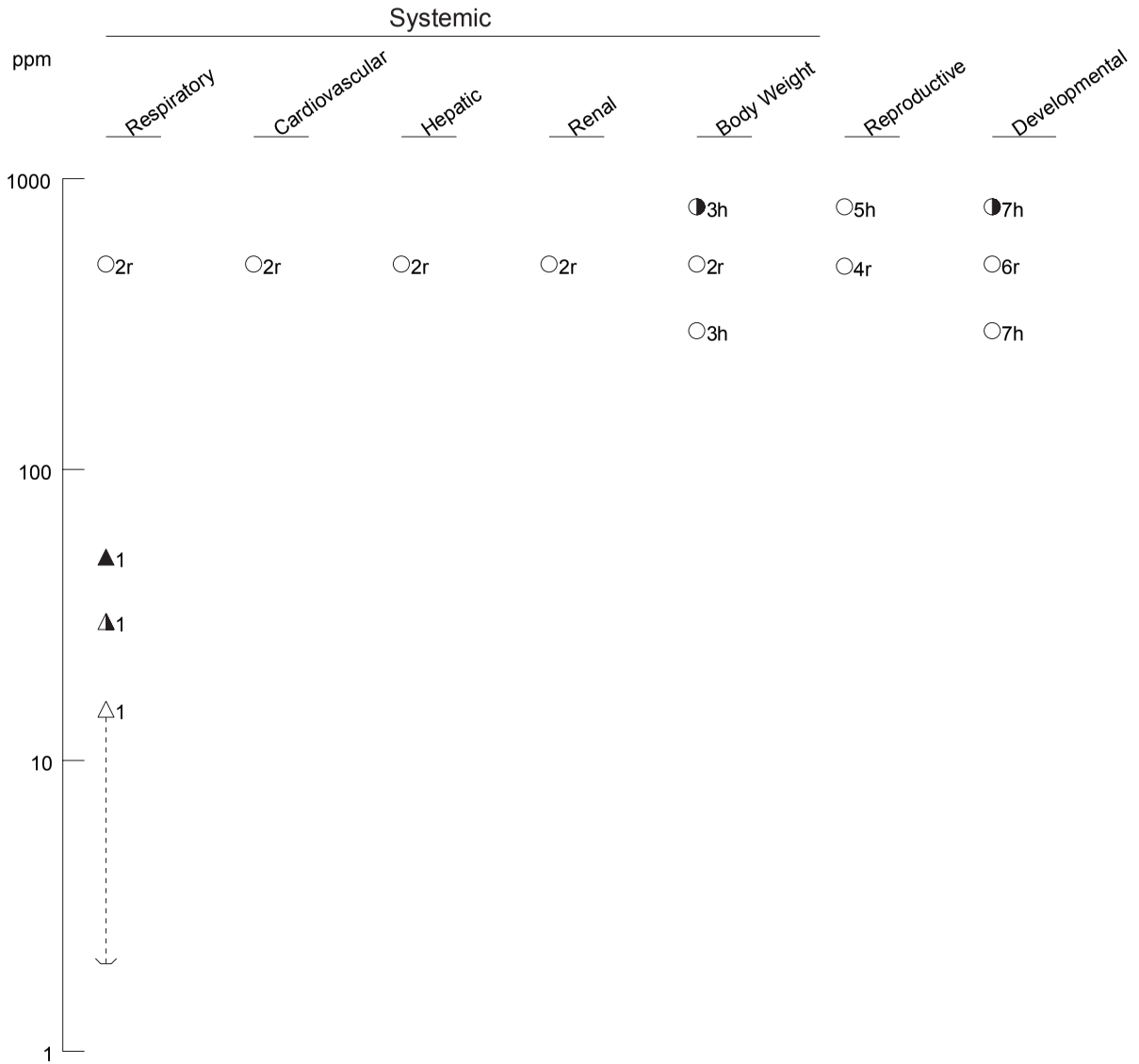
d Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

e Study result used to derive a chronic-duration inhalation Minimal Risk Level (MRL) of 0.01 ppm for 1,4-DCB, as described in detail in Appendix A. Benchmark dose analysis was performed on incidences of nasal lesions to select a point of departure, which was adjusted for intermittent exposure and converted to a Human Equivalent Concentration, then divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans using a dosimetric adjustment and 10 for human variability).

ALT = alamine aminotransferase; Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); F = Female; Gastro = gastrointestinal; Gd = gestational day; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; Resp = respiratory; wk = week(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation

Acute (≤14 days)



c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation *(Continued)*

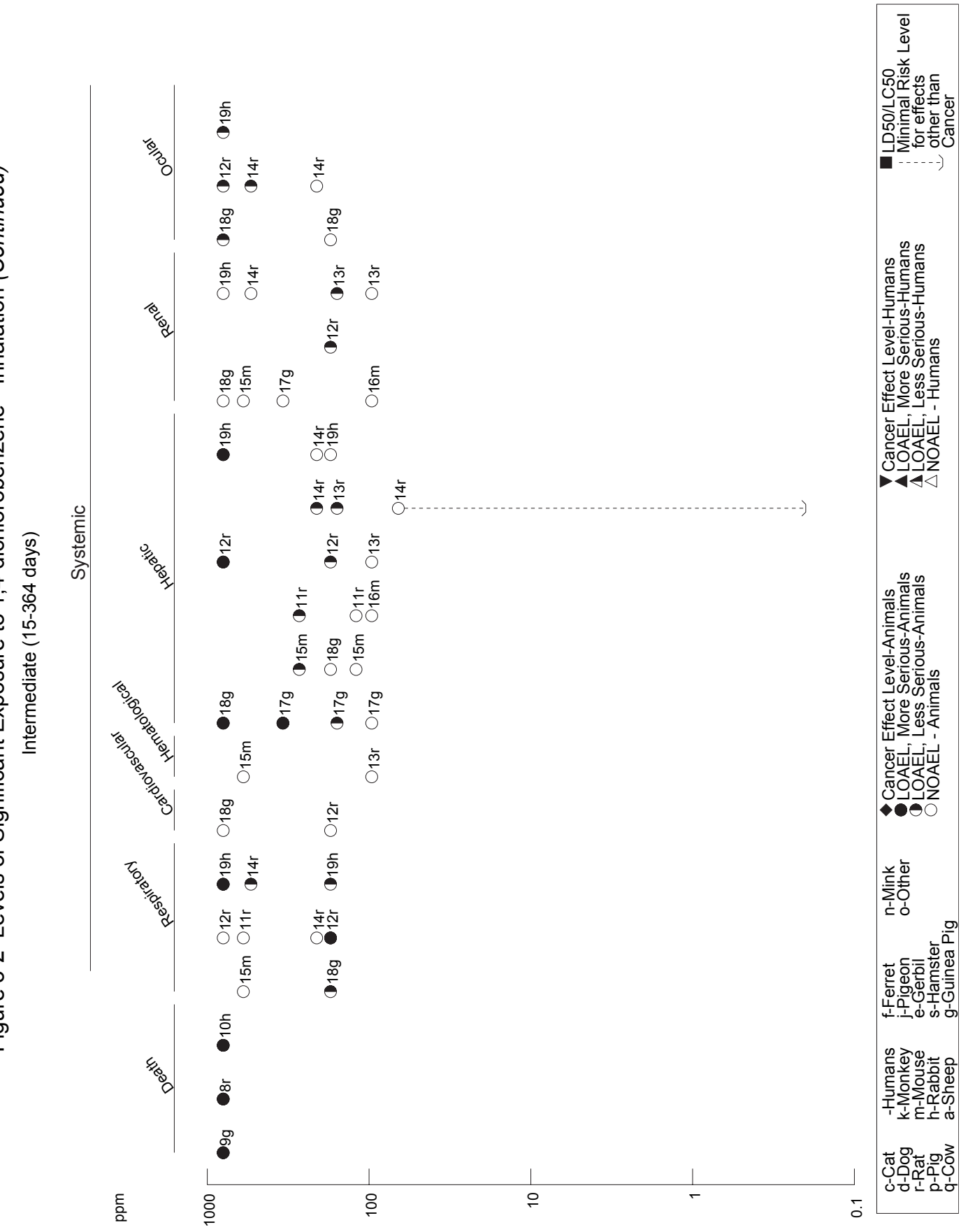


Figure 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation *(Continued)*

Intermediate (15-364 days)

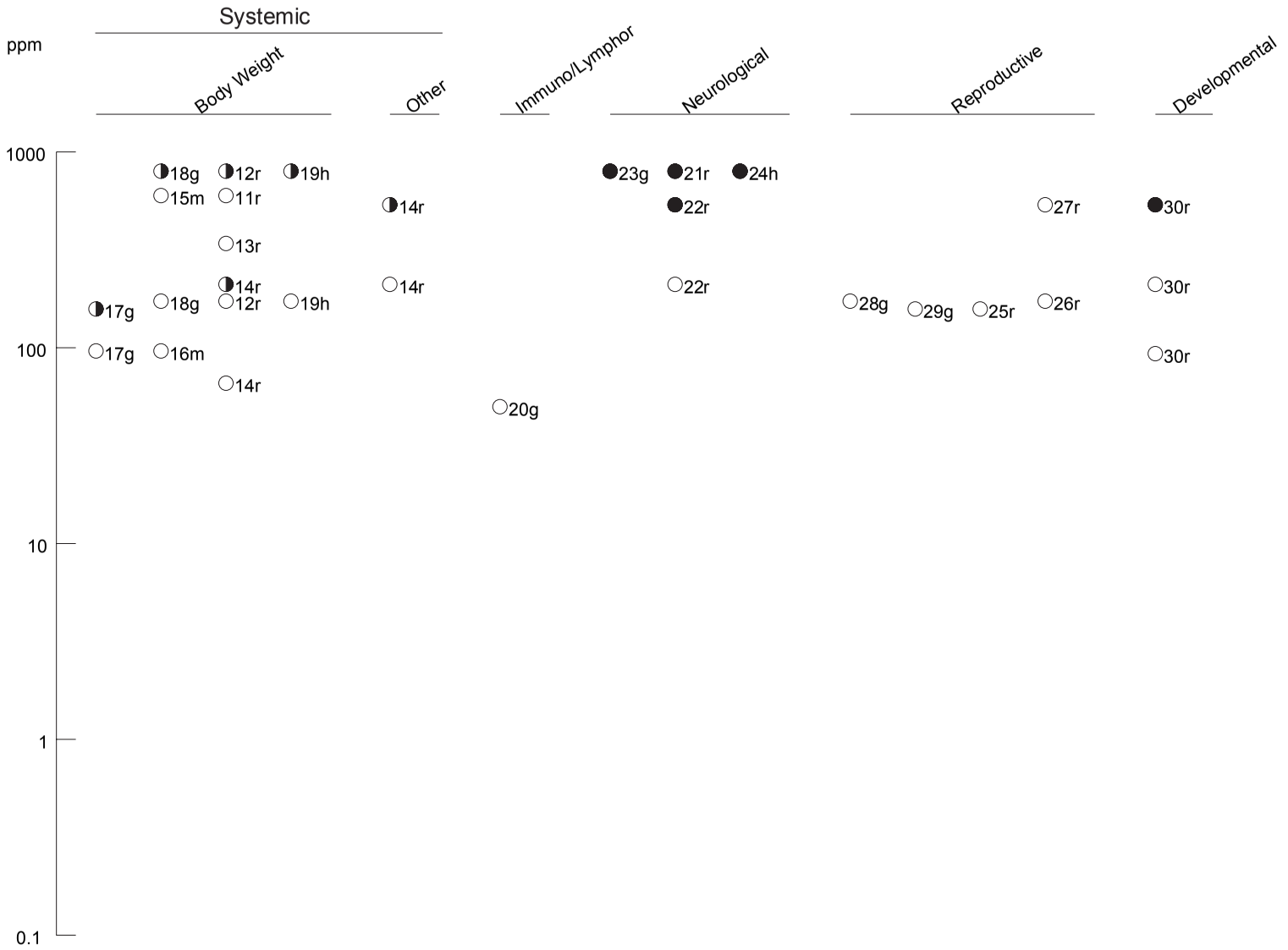
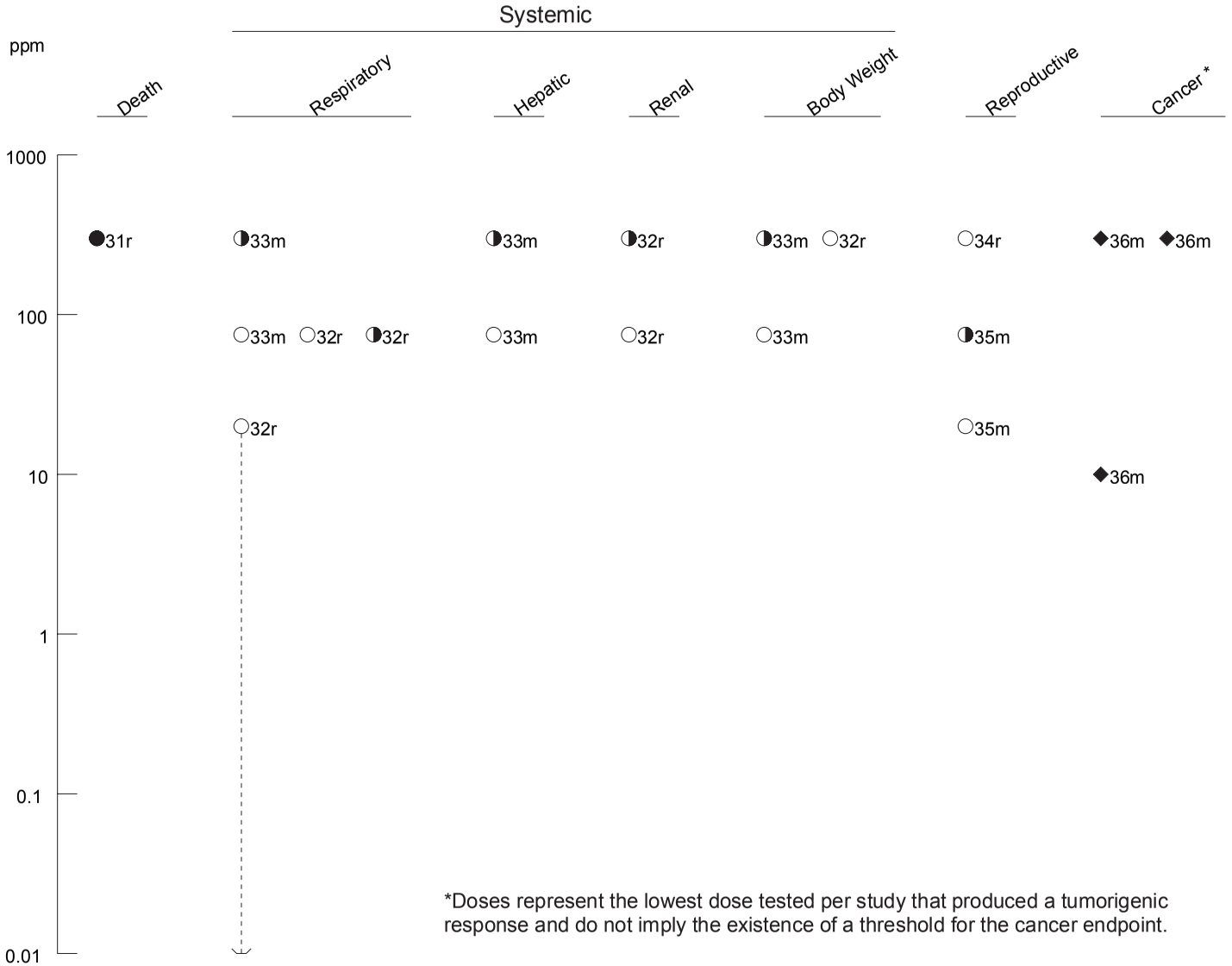


Figure 3-2 Levels of Significant Exposure to 1,4-dichlorobenzene - Inhalation

Chronic (≥365 days)



3.2.1.1 Death

1,2-Dichlorobenzene. No studies were located regarding death in humans following inhalation exposure to 1,2-DCB.

Inhalation LC_{50} values of 1,532 and 1,236 ppm were determined for rats and mice, respectively, that were exposed to 1,2-DCB for 6 hours and observed for the following 14 days (Bonnet et al. 1982). No mortality was observed in rats that were exposed to 1,2-DCB in concentrations of 977 ppm for 0.5–1 hour or 539 ppm for 3 hours (Hollingsworth et al. 1958).

1,3-Dichlorobenzene. No studies were located regarding death in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. Only one report of human death attributed to 1,4-DCB inhalation exposure has been located in the literature. A 60-year-old man and his wife died within months of each other due to acute yellow atrophy of the liver (also known as massive hepatic necrosis or fulminant hepatitis; diagnosis was not verified histologically) (Cotter 1953). Their home had been "saturated" with 1,4-DCB moth ball vapor for a period of about 3–4 months, but no air measurements were available. Clinical symptoms included severe headache, diarrhea, numbness, clumsiness, slurred speech, weight loss (50 pounds in 3 months in the case of the husband), and jaundice. The wife died within a year of the initial exposure; however, it was not clear if 1,4-DCB was the primary cause of death. This case study did not address whether these individuals consumed excessive amounts of alcohol or had previous medical problems, such as a chronic liver infection.

Several studies were located regarding death in animals after inhalation exposure to 1,4-DCB. In an acute-duration study, two of six male CD-1 mice exposed to 1,4-DCB at an air concentration of 640 ppm, 6 hours/day for 5 days died on the fifth day; no deaths were reported at an exposure level of 320 ppm (Anderson and Hodge 1976).

Mortality data were also reported in intermediate-duration studies using rats, guinea pigs, and rabbits. In studies performed by Hollingsworth et al. (1956), rats, guinea pigs, and rabbits were exposed to 1,4-DCB vapors for 9–12 weeks at an air concentration of 798 ppm, 8 hours/day, 5 days/week. In that study, 4 of 34 rats, 2 of 23 guinea pigs, and 4 of 16 rabbits died during the study period. The exact number of exposures that resulted in death was not specified.

In a chronic-duration study, there was no evidence of a treatment effect on mortality in Wistar rats exposed to 1,4-DCB at concentrations up to 490–499 ppm for 5 hours/day, 5 days/week for 76 weeks (Riley et al. 1980a).

Another chronic study found that survival was significantly reduced in male rats (F344/DuCrj) that were exposed 300 ppm 1,4-DCB for 6 hours/day, 5 days/week for 104 weeks (Aiso et al. 2005b; Japan Bioassay Research Center 1995). Survival in the male rats was noticeably lower than controls beginning at approximately study week 80, and terminal survival in the 0, 20, 75, and 300 ppm groups of the study were 66% (33/50), 68% (34/50), 58% (29/50), and 36% (18/50), respectively. There were no effects on survival in similarly exposed female rats. Male mice (Crj:BDF_1) that were similarly exposed to the same levels of 1,4-DCB had slightly reduced survival at all levels of exposure (80% [39/49], 63% [31/49], 64% [32/50], and 61% [30/49] at 0, 20, 75, and 300 ppm, respectively), but the decreases were not significantly different from controls or dose-related. Survival in female mice was similar to controls.

3.2.1.2 Systemic Effects

Respiratory Effects.

1,2-Dichlorobenzene. Periodic industrial hygiene surveys and medical examinations were conducted in a plant where an unreported number of men were exposed to 1,2-DCB at an average level of 15 ppm (range 1–44 ppm) for an unreported duration (Hollingsworth et al. 1958). No nasal or eye irritation was attributable to exposure. Additionally, Hollingsworth et al. (1958) noted that his researchers detected 1,2-DCB odor at a concentration of 50 ppm without eye or nasal irritation during repeated vapor inhalation experiments on animals. An earlier source (Elkins 1950) referenced by Hollingsworth (1958) reported that occupational exposure to 100 ppm of 1,2-DCB caused irritation of the eyes and respiratory passages.

No changes in absolute lung weight or lung histology were reported in rats (20/sex), guinea pigs (8/sex), rabbits (2/sex), or monkeys (2 females) exposed to 93 ppm 1,2-DCB for 7 hours/day, 5 days/week for 6–7 months, or in mice (10 females) similarly exposed to 49 ppm 1,2-DCB (Hollingsworth et al. 1958). Relative lung weight was not determined. The scope of histological evaluations was not specifically reported; organs that were weighed are inferred to have been histologically examined.

Histological examinations of the upper and lower respiratory tract were conducted in groups of 10 male Swiss OF1 mice that were exposed to 1,2-DCB in actual mean concentrations of 0, 64, or 163 ppm (0, 385, or 980 mg/m^3) for 6 hours/day, 5 days/week for 4, 9, or 14 days (Zissu 1995). Histological examinations were performed on the upper and lower respiratory tracts. Nonrespiratory tissues were not evaluated. Histopathologic lesions were observed in the olfactory epithelium of the nasal cavity at ≥64 ppm. The olfactory epithelial lesions were graded as very severe following the 4-day exposure and moderate after the 14-day exposure, indicating to the authors that a repair mechanism may take place despite continued exposure. The more severe cases were characterized by a complete loss of olfactory epithelium, which left only the partially denuded basement membrane. No histological alterations were observed in the respiratory epithelium of the nasal cavity, or in the trachea or lungs. The results suggest that the upper respiratory tract is a target for inhalation exposures to 1,2-DCB.

1,3-Dichlorobenzene. No studies were located regarding respiratory effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. A case of pulmonary granulomatosis was reported to have occurred in a 53-year-old woman who, for 12–15 years, had been inhaling 1,4-DCB crystals that were scattered on a weekly basis on the carpets and furniture of her home. A lung biopsy revealed the presence of 1,4-DCB crystals with the surrounding lung parenchyma being distorted by fibrosis, thickening of the alveolar walls, and marked infiltrates of lymphocytes and mononuclear phagocytes. Also, there was some thickening of the muscular walls of small arteries and focal fibrous thickening of the pleura (Weller and Crellin 1953). These effects are most likely related to the physical interaction of 1,4-DCB crystals (or any crystals when inhaled) with lung tissue, rather than to chemical toxicity. This conclusion by the authors of the study was based on exposure history of the patient, radiography, and histological examination of the lung tissue which showed the presence of birefringent crystals and a clear granulatomous reaction.

A study of 58 men occupationally exposed for 8 hours/day, 5 days/week, continually or intermittently, for 8 months to 25 years (average, 4.75 years) to 1,4-DCB found that the odor was faint at 15–30 ppm and strong at 30–60 ppm (Hollingsworth et al. 1956). Painful irritation of the nose and eyes was usually experienced at 50–80 ppm, although the irritation threshold was higher (80–160 ppm) in workers acclimated to exposure. At levels >160 ppm, the air was considered not breathable for unacclimated persons. The results of this study indicate that nose and eye irritation are critical effects of acute and repeated exposures to 1,4-DCB in humans. Because odor detection is a warning property expected to

prevent irritation caused by 1,4 DCB (Hollingsworth et al. 1956), 15 ppm was designated a NOAEL for irritant effects and used to derive an MRL of 2 ppm for acute inhalation exposure to 1,4-DCB.

Associations between blood concentrations of 1,4-DCB and 10 other volatile organic compounds (VOCs) and pulmonary function were evaluated in 953 adult participants in the Third National Health and Nutrition Examination Survey (NHANES III) (1988–1994) of the general population who had both blood VOC and pulmonary function measurements (Elliot et al. 2006). The mean age of the subjects was 36.6 years (range 20–59), 43.1% were female, and 26.3% were current smokers. Pulmonary function measures included forced expiratory volume at 1 second (FEV_1), forced vital capacity (FVC), peak expiratory flow rate (PEFR), and maximum mid-expiratory flow rate (MMEFR). Least squares regression models were used to evaluate the association between each VOC and each pulmonary function outcome. The models used natural log transformations of VOC concentrations, and were adjusted for race/ethnicity, age, standing height, body mass index, sex, smoking, and emphysema to account for differences in pulmonary function based on these characteristics. In the models unadjusted for smoking variables, reductions in at least one pulmonary function outcome were statistically significant for 1,4-DCB, benzene, ethylbenzene, styrene, and toluene. When the models were adjusted for smoking variables, 1,4-DCB was the only VOC that was statistically significantly associated with reduced pulmonary function. Among all 1,4-DCB participants (n=846), there was a statistically significant ($p<0.05$) inverse relationship between 1,4-DCB level and FEV_1 and MMEFR. The linear regression coefficient (β) was -96 mL (95% CI -182 to -11) for FEV_1 and -198 mL/sec (95% CI -388 to -8) for MMEFR. The β coefficient estimates the expected change in lung function as the concentration of 1,4-DCB increases from the 10^{th} to 90^{th} percentile (3.76 µg/L) on the natural log scale. Analysis by race and sex showed statistically significant results for FEV_1 in non-Hispanic white females [β=-266 mL (95% CI -488 to -43)] and African-American males [β=-282 mL (95% CI -497 to -66)]. Analyses conducted in 534 subjects using urinary concentrations of 1,4-DCB and its major metabolite, 2,5-dichlorophenol, showed statistically significant β coefficients for FEV_1 for both 1,4-DCB (-96 mL, 95% CI not reported) and 2,5-dichlorophenol (-134 mL, 95% CI not reported). Analyses were also performed using non-logarithmically transformed blood concentrations of 1,4-DCB that were categorized into deciles. Tests for linear trend across deciles were statistically significant for FEV_1 and MMEFR. Compared with subjects in the lowest decile of 1,4-DCB concentration (0.10 ppb), subjects in the highest decile (>4.40 ppb) had FEV_1 decrements of -153 mL (95% CI -297 to -8) and MMEFR decrements of -346 mL/sec (95% CI -667 to -24). The authors concluded that the findings of this study suggest that exposure to 1,4-DCB at levels found in the general population may result in decreases in lung function.

In pregnant Alderley-Park rats, whole-body exposure to 1,4-DCB at air concentrations of 74.7, 198.6, or 508.4 ppm, 6 hours/day on gestation days (Gd) 6–15 produced no adverse clinical or pathological signs in the lung tissues of the dams (Hodge et al. 1977). Mild histopathological changes of interstitial edema, congestion, and alveolar hemorrhage were observed in the lungs of male (but not female) rats, female guinea pigs, and one female rabbit after 16 days of exposure to 1,4-DCB at 173 ppm (Hollingsworth et al. 1956). Congestion and emphysema were also reported in the lungs of two rabbits exposed to 798 ppm for 12 weeks (Hollingsworth et al. 1956). These observations were derived from a large study using several species of laboratory animals; however, interspecies comparisons are difficult to make due to the various experimental designs used in this study. For example, at 798 ppm, 10 male rats, 15 female rats, 16 male guinea pigs, seven female guinea pigs, and 8 rabbits of each sex were exposed up to 62 times; at 173 ppm, five rats of each sex, five guinea pigs of each sex, and one rabbit of each sex were exposed for 16 days. These reported observations provide only qualitative evidence of respiratory effects as a result of intermediate-duration inhalation exposure to 1,4-DCB.

An intermediate-duration study was conducted in which F344 rats and BDF_1 mice were chamber–exposed to 25, 55, 120, 270, or 600 ppm of 1,4-DCB for 6 hours/day, 5 days/week for 13 weeks (Aiso et al. 2005a). No histological changes in the respiratory tract were reported. This study apparently conformed to (OECD) (1981) testing guidelines for a 90-day inhalation toxicity study, indicating that the histological examinations included naso-pharyngeal tissues and lungs.

In a chronic-duration study, male and female Wistar rats were exposed to 1,4-DCB at air concentrations of 75 or 490–499 ppm, 5 hours/day, 5 days/week for 76 weeks (Riley et al. 1980a). Rats in the high-exposure group showed a small but significant increase in absolute lung weight at termination of the study (112 weeks). This response was not observed in rats sacrificed on week 76 or in rats exposed to 75 ppm 1,4-DCB for 112 weeks. No treatment-related histological alterations were observed in the larynx, trachea, or lungs in this study.

Another chronic inhalation study was conducted in which groups of 50 male and female F344/DuCrj rats, and 50 male and 50 female Crj:BDF_1 mice, were exposed to 1,4-DCB in concentrations of 0, 20, 75, or 300 ppm for 6 hours/day, 5 days/week for 104 weeks (Aiso et al. 2005b; Japan Bioassay Research Center 1995). Histological examinations of the respiratory tract (nasal cavity, trachea, and lung) showed nasal epithelial effects in rats and mice. The nasal lesions in rats mainly included eosinophilic changes of moderate or greater severity in the olfactory epithelium in male rats at 300 ppm and female rats at ≥75 ppm. Incidences of this lesion at 0, 20, 75, and 300 ppm were 1/50, 2/50, 2/50, and 7/50 in the male

rats, and 28/50, 29/50, 39/50, and 47/50 in the female rats. The increases were significantly ($p \leq 0.05$) different than the control values and there was a trend of increasing response with increasing dose in both sexes. Additionally observed were significantly increased incidences of eosinophilic changes of the respiratory epithelium and respiratory metaplasia in the 300 ppm female rats only. The nasal lesions in mice included significantly increased incidences of respiratory metaplasia in the nasal gland (moderate severity) in males at 75 ppm (9/49, 12/49, 18/50, 11/49) and olfactory epithelium (slight severity) in males at 75 ppm (23/49, 30/49, 37/50, 22/49) and females at 300 ppm (7/50, 6/50, 2/49, 20/50), but the effects in the males were not dose-related (i.e., incidences were increased at 75 ppm but not at 300 ppm). The nasal lesions in female rats, the more sensitive species and sex, were selected as the critical effect for deriving a chronic-duration inhalation MRL of 0.01 ppm for 1,4-DCB.

Cardiovascular Effects.

1,2-Dichlorobenzene. No studies were located regarding cardiovascular effects in humans following inhalation exposure to 1,2-DCB.

No changes in absolute heart weight or heart histology were reported for rats (20/sex), guinea pigs (8/sex), rabbits (2/sex), or monkeys (2 females) following exposure to 93 ppm 1,2-DCB for 7 hours/day, 5 days/week for 6–7 months, or in mice (10 females) that were similarly exposed to 49 ppm 1,2-DCB (Hollingsworth et al. 1958). Relative heart weight was not determined. The scope of histological evaluations was not specifically reported; organs that were weighed are inferred to have been histologically examined.

1,3-Dichlorobenzene. No studies were located regarding cardiovascular effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding cardiovascular effects in humans following inhalation exposure to 1,4-DCB.

Limited information is available regarding cardiovascular effects in animals. No alterations in relative heart weight were observed in rats or guinea pigs exposed to 1,4-DCB at an air concentration of 173 ppm, 7 hours/day, 5 days/week for up to 12 exposures (Hollingsworth et al. 1956). Similar results were reported after approximately 130 exposures to 1,4-DCB at an air concentration of 96 ppm using the same

exposure protocol (Hollingsworth et al. 1956); no other cardiovascular end points were evaluated in this study.

In pregnant Alderley-Park rats, whole-body exposure to 1,4-DCB at air concentrations of 74.7, 198.6, or 508.4 ppm, 6 hours/day from Gd 6 to 15 produced no adverse clinical or pathological signs in the heart tissues of the dams (Hodge et al. 1977).

A significant increase in absolute heart weight was reported in male and female rats exposed to 1,4-DCB at air concentrations of 490–499 ppm, 5 hours/day, 5 days/week for 76 weeks and allowed to recover until week 112 (Riley et al. 1980a). This effect was not seen at the 76-week interim sacrifice or at the lower-exposure concentration of 75 ppm. Examination of the heart and aorta at interim sacrifices or at termination of the study revealed no significant histological alterations related to 1,4-DCB treatment.

Gastrointestinal Effects.

1,2-Dichlorobenzene. No studies were located regarding gastrointestinal effects in humans or animals following inhalation exposure to 1,2-DCB.

1,3-Dichlorobenzene. No studies were located regarding gastrointestinal effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. Two case reports provide evidence of gastrointestinal effects in humans after exposure to unknown concentrations of 1,4-DCB. A 60-year-old man who had been exposed to vapors of 1,4-DCB in his home for 3–4 months reported having several bowel movements a day with loose tarry stools for 10 days before being admitted to a hospital (Cotter 1953). The second case is that of a 34-year-old woman who had been exposed to vapors of 1,4-DCB at work and became acutely ill with nausea and vomiting, and was hospitalized with hemorrhage from the gastrointestinal tract (Cotter 1953). The physical and chemical findings led to the diagnosis of subacute yellow atrophy and cirrhosis of the liver from 1,4-DCB exposure. No further information was located.

Limited information regarding gastrointestinal effects in animals is provided in a chronic-duration study. In that study (Riley et al. 1980a), the investigators found no effect on the organ weight or on gross and histopathological appearance of the caecum, colon, duodenum, jejunum, esophagus, pancreas, and

stomach in male and female Wistar rats exposed to 1,4-DCB at air concentrations of up to 490–499 ppm, 5 hours/day, 5 days/week for 76 weeks.

Hematological Effects.

1,2-Dichlorobenzene. Periodic industrial hygiene surveys and medical examinations were conducted in a plant where an unreported number of men were exposed to 1,2-DCB at an average level of 15 ppm (range 1–44 ppm) for an unreported duration (Hollingsworth et al. 1958). No effects on clinical hematology indices (red blood cell count, total and differential white blood cell counts, hemoglobin, hematocrit, and mean corpuscular volume) were attributable to exposure.

Red blood cell (RBC), total white blood cell (WBC), and leucocyte differential cell counts were assessed in groups of five male Sprague-Dawley rats that were exposed to 0, 5, 10, 16, or 29 ppm 1,2-DCB for 4 hours (Brondeau et al. 1990). Total WBC counts were significantly ($p \leq 0.05$) reduced at ≥10 ppm without any changes in WBC differential or RBC counts. The effect of 1,2-DCB on total WBC count was further assessed in groups of 10 male Sprague-Dawley rats that were normal or adrenalectomized and exposed to 0 or 24 ppm for 4 hours. Adrenalectomy caused a significant increase in total WBCs (39.9% higher than normal controls), although exposure did not significantly affect WBC count in the adrenalectomized rats. Because the adrenal-dependent leucopenia was similar to that observed after exposure to various irritant stressors, and is thought to be a secondary manifestation of increased secretion of glucocorticosteroids, the authors considered the effect to be an associative response to sensory irritation.

No hematological changes were reported in rabbits (2/sex) or monkeys (2 females) that were exposed to 93 ppm 1,2-DCB for 7 hours/day, 5 days/week for 6–7 months (Hollingsworth et al. 1958). The hematology end points that were evaluated were not specified.

1,3-Dichlorobenzene. No studies were located regarding hematological effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. Two reports of hematological effects in humans after inhalation exposure to 1,4-DCB were located in the literature. Based on results from blood counts, anemia was diagnosed in two men; one had been exposed to unknown concentrations of 1,4-DCB vapors at home for 3–4 months and the other had been in a storage plant saturated with 1,4-DCB vapor. A woman exposed in a similar

manner was diagnosed with borderline anemia (Cotter 1953). Early industrial hygiene surveys found no evidence of adverse hematological effects attributable to exposure to 1,4-DCB in workers at air concentrations ranging from 10 to 550 ppm for 8 months to 25 years (average 4.75 years) (Hollingsworth et al. 1956).

Information regarding hematological effects in animals is scant. No hematologic effects (specific tests not provided) were observed in rats and rabbits exposed to 1,4-DCB vapors at concentrations of 96 or 158 ppm, respectively, dosed for durations of 7 hours/day, 5 days/week for 5–7 months (Hollingsworth et al. 1956). In another intermediate-duration study, F344 rats and BDF_1 mice were chamber-exposed to 25, 55, 120, 270, or 600 ppm of 1,4-DCB for 6 hours/day, 5 days/week for 13 weeks (Aiso et al. 2005a). Hematological changes suggestive of microcytic anemia occurred in the male rats; effects included significantly decreased RBC count and hemoglobin concentration at ≥120 ppm, hematocrit at ≥270 ppm, and MCV and MCH at 600 ppm. The effects were not accompanied by any anemia-associated histopathological changes in hematopoietic tissues (e.g., increased extramedullary hematopoiesis or hemosiderosis in the spleen) and did not occur in the female rats or mice of either sex, leading the investigators to suggest that they were secondary to male rat-specific $\alpha_{2\mu}$-globulin nephropathy-related effects on erythropoietin synthesis in the renal tubules.

A chronic-duration study reported that some changes in blood chemistry and hematologic parameters were seen in rats exposed 5 hours/day, 5 days/week to 1,4-DCB at air concentrations of up to 490–499 ppm for 76 weeks; however, the reported changes showed no consistent trend with dose, sex, or exposure duration that would indicate treatment-related effects (Riley et al. 1980a).

Musculoskeletal Effects.

1,2-Dichlorobenzene. No studies were located regarding musculoskeletal effects in humans following inhalation exposure to 1,2-DCB.

1,3-Dichlorobenzene. No studies were located regarding musculoskeletal effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding musculoskeletal effects in humans after inhalation exposure to 1,4-DCB.

One study was located that examined the musculoskeletal effects in laboratory animals after inhalation exposure to 1,4-DCB. No gross or histological alterations in skeletal muscle (unspecified parameters) were detected in rats exposed to 1,4-DCB at air concentrations of up to 490–499 ppm, 5 hours/day, 5 days/week for 76 weeks (Riley et al. 1980a).

Hepatic Effects.

1,2-Dichlorobenzene. No studies were located regarding hepatic effects in humans following inhalation exposure to 1,2-DCB.

Increased liver weight and marked central lobular necrosis occurred in rats that were exposed to 1,2-DCB at a concentration of 977 ppm for 0.5 or 1 hour, but not to 539 ppm for 3 hours (Hollingsworth et al. 1958). No changes in absolute liver weight or hepatic histology were reported for rats (20/sex), guinea pigs (8/sex), rabbits (2/sex), or monkeys (2 females) exposed to 93 ppm 1,2-DCB for 7 hours/day, 5 days/week for 6–7 months, or in mice (10 females) similarly exposed to 49 ppm 1,2-DCB (Hollingsworth et al. 1958).

1,3-Dichlorobenzene. No studies were located regarding hepatic effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. Hepatic effects have been reported in humans following long-term exposure to 1,4-DCB via inhalation. A 60-year-old man and his wife who were exposed to moth ball vapor that "saturated" their home for 3–4 months both died of liver failure (acute liver atrophy) within a year of the initial exposure (Cotter 1953). Yellow atrophy and cirrhosis of the liver were reported in a 34-year-old woman who demonstrated 1,4-DCB products in a department store and in a 52-year-old man who used 1,4-DCB occupationally in a fur storage plant for about 2 years (Cotter 1953). Duration of exposure was not estimated for the 34-year-old woman, but was indicated in the report to be >1 year. No estimates of the 1,4-DCB exposure levels (other than the use of the term “saturated”) were provided in any of these reports, nor was it verified that 1,4-DCB exposure was the only factor associated with the observed effects. History of alcohol consumption or prior liver disease factors were not mentioned for any of the cases reported by Cotter (1953). These case studies indicate that the liver is a target organ for 1,4-DCB in humans, but they do not provide quantitative information.

In an acute-duration study using pregnant Alderley-Park rats, whole-body exposure to 1,4-DCB at air concentrations of 74.7, 198.6, or 508.4 ppm, 6 hours/day from Gd 6 to 15 produced no adverse clinical or pathological signs in the hepatic tissues of the dams (Hodge et al. 1977). In a similar study, New Zealand White rabbits exposed whole-body to 1,4-DCB 6 hours/day on Gd 6–18 experienced no adverse effects on absolute or relative maternal liver weights at air concentrations up to 800 ppm (Hayes et al. 1985).

An intermediate-duration study was conducted in which F344 rats and BDF_1 mice were chamber-exposed to 0, 25, 55, 120, 270, or 600 ppm of 1,4-DCB for 6 hours/day, 5 days/week for 13 weeks (Aiso et al. 2005a). Hepatic effects in the rats included increases in absolute and relative liver weight (>10%) in males at ≥270 ppm and females at 600 ppm, serum total cholesterol and phospholipid in males at ≥270 ppm and females at 600 ppm, serum albumin in females at ≥270 ppm and males at 600 ppm, total protein in both sexes at 600 ppm, and centrilobular hepatocellular hypertrophy in males at 600 ppm. Hepatic effects in the mice included increases in absolute and relative liver weight (>10%) in males at ≥270 ppm and females at 600 ppm, serum ALT in males at ≥270 ppm and females at 600 ppm, serum AST in males at 600 ppm, serum total cholesterol and total protein in both sexes at 600 ppm, and centrilobular hepatocellular hypertrophy in males at ≥270 ppm and females at 600 ppm. The mouse liver was more responsive to 1,4-DCB than the rat liver as shown by the histological and serum enzyme changes. Hepatocellular hypertrophy occurred at a lower exposure level in the mice (270 ppm compared to 600 ppm in rats); incidences in the 0, 25, 120, 270, and 600 ppm male mice were 0/10, 0/10, 0/10, 0/10; 10/10 and 10/10, respectively. At 600 ppm, the severity of the hepatocellular hypertrophy was classified as moderate in the mice and slight in the rats. Affected hepatocytes in the mice were characterized by cell enlargement, varying nuclear size and shape, and coarse chromatin and inclusion bodies in the nucleus, whereas such nuclear changes were not observed in the hypertrophic hepatocytes of the rats. Additionally, the hepatocellular hypertrophy in the mice was accompanied by single cell necrosis (both sexes, incidence not reported) and focal necrosis (2/10 males) at 600 ppm, as well as the increases in serum ALT at ≥270 ppm and AST at 600 ppm, whereas none of these indicators of hepatocellular damage occurred in the rats.

In a cross-species comparative study, exposure to 1,4-DCB at air concentrations up to 158 ppm, 7 hours/day, 5 days/week for 5–7 months produced no treatment-related effects on liver weight or microscopic appearance in male and female mice; in contrast, various hepatic effects were noted in rats, guinea pigs, and rabbits exposed to 1,4-DCB at various levels and durations of exposure (Hollingsworth et al. 1956). There was considerable variability in the species of animals exposed at each dose, the number of animals exposed, and the total number of exposures. When rats and rabbits inhaled 173–

798 ppm of 1,4-DCB intermittently for 2–12 weeks, several hepatic effects were observed. Relative liver weight was increased in rats exposed to 173 ppm; histopathological examination at this exposure level revealed slight congestion and granular degeneration in female rats. At 798 ppm, liver changes included cloudy swelling and central necrosis in both sexes of rats and rabbits. In the same study, when rats inhaled 158–341 ppm 1,4-DCB intermittently for 5–7 months, male and female rats displayed cloudy swelling and central zone degeneration of the hepatic parenchymal cells in the liver, and increased relative liver weights at 158 ppm. These changes were not seen at a concentration of 96 ppm. In the same study, guinea pigs that were exposed to 341 ppm for a comparable duration or to 798 ppm for 2–4.5 weeks had focal necrosis and slight cirrhosis (in some animals) as well as hepatocyte swelling and degeneration.

In a 2-generation study of the effects of inhalation exposure to 1,4-DCB in Sprague-Dawley rats, males and females were exposed to 0, 66.3, 211, or 538 ppm 1,4-DCB 6 hours/day for 10 weeks prior to mating. The females were also exposed during mating, and on Gd 0–19 and postnatal days 5–27; males were exposed throughout the study. Marked hepatocellular hypertrophy, localized in the centrilobular area, was noted in F_0 and F_1 males and females in the 538 ppm dose group; no such effects were seen in the low- and mid-dose groups. Liver weights were significantly elevated in F_0 males at the 211 and 538 ppm doses and in F_0 females at the 538 ppm dose; liver weights were also significantly elevated in F_1 males and females at the 538 ppm dose (Tyl and Neeper-Bradley 1989). The increased liver weight in F_0 male rats was selected as the critical effect for deriving an intermediate-duration inhalation MRL of 0.2 ppm for 1,4-DCB.

In a long-term inhalation study in rats, exposure to 1,4-DCB at air concentrations of 490–499 ppm 5 hours/day, 5 days/week for 76 weeks resulted in an increase in absolute liver weight throughout the study in males and at weeks 27 and 112 in females (Riley et al. 1980a). This effect was not accompanied by histological alterations or by increased serum transaminase activities. No hepatic effects were noted at 75 ppm. None of the adverse hepatic effects reported at lower concentrations of 1,4-DCB for shorter durations (Hollingsworth et al. 1956), as described above, were identified in the 76-week study.

In another chronic study, groups of 50 male and female F344/DuCrj rats and 50 male and 50 female Crj:BDF_1 mice were exposed to 1,4-DCB in concentrations of 0, 20, 75, or 300 ppm for 6 hours/day, 5 days/week for 104 weeks (Aiso et al. 2005b; Japan Bioassay Research Center 1995). Histological examinations showed liver changes only in the high-dose male mice. The incidence of centrilobular

hepatocellular hypertrophy was significantly increased in male mice at 300 ppm, as shown by incidences of 0/49, 0/49, 0/50, and 34/49 in the control to high dose groups.

Renal Effects.

1,2-Dichlorobenzene. Periodic industrial hygiene surveys and medical examinations were conducted in a plant where an unreported number of men were exposed to 1,2-DCB at an average level of 15 ppm (range 1–44 ppm) for an unreported duration (Hollingsworth et al. 1958). No effects on clinical renal indices (blood urea nitrogen, sedimentation rate, or urinalysis) were attributable to exposure.

No changes in absolute kidney weight or kidney histology were reported for rats (20/sex), guinea pigs (8/sex), rabbits (2/sex), or monkeys (2 females) exposed to 93 ppm 1,2-DCB for 7 hours/day, 5 days/week for 6–7 months, or in mice (10 females) similarly exposed to 49 ppm 1,2-DCB (Hollingsworth et al. 1958). Relative kidney weight was not determined. The scope of histological evaluations was not specifically reported; organs that were weighed are inferred to have been histologically examined. Limited urinalysis was performed in the species exposed to 93 ppm; BUN determinations and qualitative tests for sugar, albumin, sediment, and blood showed no abnormalities.

1,3-Dichlorobenzene. No studies were located regarding renal effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding renal effects in humans after inhalation exposure to 1,4-DCB.

In an acute-duration study using pregnant Alderley-Park rats, whole-body exposure to 1,4-DCB at air concentrations of 74.7, 198.6, or 508.4 ppm, 6 hours/day from Gd 6 to 15 produced no adverse clinical or pathological signs in the kidney tissues of the dams (Hodge et al. 1977). In a similar study, pregnant New Zealand White rabbits exposed whole-body to 1,4-DCB 6 hours/day on Gd 6–18 experienced no adverse effects with regard to either absolute or relative maternal kidney weights at air concentrations up to 800 ppm (Hayes et al. 1985).

In an intermediate-duration study, F344 rats and BDF_1 mice were chamber-exposed to 25, 55, 120, 270, or 600 ppm of 1,4-DCB for 6 hours/day, 5 days/week for 13 weeks (Aiso et al. 2005a). Histological effects included kidney lesions indicative of $\alpha_{2\mu}$-globulin nephropathy (hyaline droplets, granular casts,

tubular cell necrosis, cytoplasmic basophila, and papillary mineralization) in the male rats at ≥270 ppm. There were no histological changes in the kidneys of the female rats or mice of either sex. Other renal effects included increased relative and/or absolute kidney weights in male rats and male mice at ≥270 ppm and female rats and female mice at 600 ppm, and increased serum BUN in male rats and male mice at 600 ppm.

In rats, mice, and rabbits exposed by inhalation to 1,4-DCB at air concentrations ranging from 96 to 798 ppm, 7 or 8 hours/day, for periods as long as 7 months, no renal effects were noted in mice or rabbits, while both male and female rats experienced increased relative kidney weights at the 173 ppm dose level. In addition, a slight cloudy swelling of the tubular epithelium was noted in female rats exposed to 798 ppm. In the same study, inhalation of 1,4-DCB at 158 or 341 ppm intermittently for 5–7 months by rats caused a slight increase in relative kidney weight in males but not females (Hollingsworth et al. 1956). This effect was not observed in groups of guinea pigs, in one monkey, or in two rabbits under the same experimental conditions (Hollingsworth et al. 1956). The findings in this study are consistent with those reported by Riley et al. (1980a) in a 76-week study in rats, described below.

In a 2-generation study of the effects of inhalation exposure to 1,4-DCB in Sprague-Dawley rats, males and females were exposed to 0, 66.3, 211, or 538 ppm 1,4-DCB 6 hours/day for 10 weeks prior to mating. The females were also exposed during mating, and on Gd 0–19 and postnatal days 5–27; males were exposed throughout the study. An increased incidence of nephrosis was seen in F_0 males of all dose groups and in F_1 males of the 211 and 538 ppm dose groups; lesions consisted of hyaline droplets, tubular protein nephrosis, granular cast formation, and interstitial nephritis. No renal lesions were noted in F_0 or F_1 females. Kidney weights were significantly elevated in F_0 males at all doses and in F_1 males at the 538 ppm dose. In females, kidney weights were significantly elevated in the F_0 generation at the 538 ppm dose, but were not elevated in the F_1 generation (Tyl and Neeper-Bradley 1989).

In a chronic-duration inhalation study in Wistar rats, exposure to 1,4-DCB at air concentrations of 490–499 ppm, 5 hours/day, 5 days/week for 76 weeks resulted in an increase in absolute kidney weight in males throughout the study and in females at weeks 27 and 112 weeks. Exposure to 75 ppm 1,4-DCB had no effect on kidney weight, and neither exposure level caused histopathological alterations in the kidneys (Riley et al. 1980a). In another chronic study, groups of 50 male and female F344/DuCrj rats and 50 male and 50 female Crj:BDF_1 mice were exposed to 1,4-DCB in concentrations of 0, 20, 75, or 300 ppm for 6 hours/day, 5 days/week for 104 weeks (Aiso et al. 2005b; Japan Bioassay Research Center 1995). Histological examinations showed kidney changes only in male rats at 300 ppm, where incidences of

mineralization of the renal papilla and hyperplasia of the urothelium were significantly increased. In general, the renal effects observed in inhalation studies of 1,4-DCB are mild in contrast with the severe renal effects observed in oral studies as described in Section 3.2.2.2.

Endocrine Effects.

1,2-Dichlorobenzene. No studies were located regarding endocrine effects in humans or animals following inhalation exposure to 1,2-DCB.

1,3-Dichlorobenzene. No studies were located regarding endocrine effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding endocrine effects in humans following inhalation exposure to 1,4-DCB.

The only information regarding endocrine effects in animals after inhalation exposure to 1,4-DCB is from a chronic-duration study in rats. In that study (Riley et al. 1980a), no gross or histopathological effects were observed in the adrenal, thyroid, or pituitary glands of male or female rats exposed to 1,4-DCB at air concentrations up to 490–499 ppm, 5 hours/day, 5 days/week for 76 weeks. No further information regarding endocrine effects was located.

Dermal Effects.

1,2-Dichlorobenzene. No studies were located regarding dermal effects in humans or animals following inhalation exposure to 1,2-DCB.

1,3-Dichlorobenzene. No studies were located regarding dermal effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. Dermal effects resulting from 1,4-DCB exposure were reported in a 69-year-old man who had been exposed for approximately 3 weeks to 1,4-DCB used in his home, including on a chair on which he had been sitting. He gradually developed petechiae (small red spots), purpura (purple or brownish-red spots), and swelling of his hands and feet. His sensitivity to 1,4-DCB was established by an

indirect basophil degranulation test that showed a strongly positive reaction (degenerative changes in 62% of his basophils when tested with 1,4-DCB, compared with a 6% reaction of normal serum with 1,4-DCB) (Nalbandian and Pearce 1965). The authors suggested that these effects were probably immunologically mediated. In a study of 58 men occupationally exposed to up to 725 ppm 1,4-DCB, 8 hours/day, 5 days/week continually or intermittently for 8 months to 25 years (average: 4.75 years), medical examinations revealed no evidence of dermatological effects (Hollingsworth et al. 1956).

No studies were located regarding dermal effects in animals after inhalation exposure to 1,4-DCB.

Ocular Effects.

1,2-Dichlorobenzene. Periodic industrial hygiene surveys and medical examinations were conducted in a plant where an unreported number of men were exposed to 1,2-DCB at an average level of 15 ppm (range 1–44 ppm) for an unreported duration (Hollingsworth et al. 1958). No eye or nasal irritation was attributable to exposure. Additionally, Hollingsworth et al. (1958) noted that his researchers detected 1,2-DCB odor at a concentration of 50 ppm without eye or nasal irritation during repeated vapor inhalation experiments on animals. An earlier source (Elkins 1950) referenced by Hollingsworth (1958) reported that occupational exposure to 100 ppm of 1,2-DCB caused irritation of the eyes and respiratory passages.

1,3-Dichlorobenzene. No studies were located regarding ocular effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. In a report on 58 men who had worked for 8 months to 25 years (average exposure 4.75 years) in a plant that used 1,4-DCB, painful irritation of the nose and eyes were reported at levels ranging from 80 to 160 ppm (Hollingsworth et al. 1956). At levels >160 ppm, the air was considered unbreathable by unacclimated persons. Neither cataracts nor any other lens changes were found upon examination of their eyes.

There is no clear, quantitative evidence of ocular effects resulting from inhalation exposure to 1,4-DCB in animal studies. Ocular effects, described as reversible, nonspecific eye ground changes (changes in the fundus or back of the eye), were seen in two rabbits exposed to 1,4-DCB at 798 ppm, 8 hours/day, 5 days/week for 12 weeks (Hollingsworth et al. 1956). In the same study, no lens changes were observed in rats or guinea pigs exposed to 798 ppm 1,4-DCB, but eye irritation was reported in the three species

tested. Ocular effects occurring during and/or after exposure to chemicals in air are likely to be due to direct contact of the chemical with the eye.

A chronic-duration inhalation study in male and female Wistar rats reported no histopathological alterations in the eyes of rats exposed to 1,4-DCB at air concentrations up to 490–499 ppm, 5 hours/day, 5 days/week for 76 weeks (Riley et al. 1980a). No further data were located.

Body Weight Effects.

1,2-Dichlorobenzene. Groups of male and female albino rats (20/sex) were exposed to 0, 49, or 93 ppm (0, 290, or 560 mg/m^3, respectively) of 1,2-DCB (99% pure) vapor for 7 hours/day, 5 days/week for 6–7 months (Hollingsworth et al. 1958). No compound related effects were found at 49 ppm. Effects observed at 93 ppm consisted of statistically significant ($p \leq 0.05$) decreased final body weight in the males (8.9% lower than controls). There were no body weight changes in guinea pigs (8/sex), rabbits (2/sex), or monkeys (2 females) similarly exposed to 93 ppm 1,2-DCB, or in mice (10 females) similarly exposed to 49 ppm 1,2-DCB (Hollingsworth et al. 1958).

1,3-Dichlorobenzene. No studies were located regarding body weight effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. A 60-year-old man who was exposed to vapors of 1,4-DCB in his home for 3–4 months was reported to have lost approximately 50 pounds in body weight in 3 months (Cotter 1953). His wife, who received similar exposure, also lost weight. A third case reported by the same author (Cotter 1953) is that of a 52-year-old man who was exposed to 1,4-DCB by using the chemical for preserving raw furs. On examination, this individual was described as being emaciated. Information regarding food consumption was not available in any of these cases. In the case of the 60-year-old man, persistent diarrhea may have contributed to the weight loss.

In an acute-duration study using pregnant Alderley-Park rats, whole-body exposure to 1,4-DCB at air concentrations of 74.7, 198.6, or 508.4 ppm, 6 hours/day from Gd 6 to 15 had no effect on maternal body weight gain (Hodge et al. 1977).

Body weight data are available for various animal species after exposure to 1,4-DCB 7–8 hours/day, 5 days/week, for periods ranging from 2 weeks to 6 months (Hollingsworth et al. 1956). Rats, rabbits,

and guinea pigs experienced weight loss when exposed to 798 ppm, 8 hours/day, 5 days/week. Rats exposed to up to 341 ppm 1,4-DCB for 5–7 months grew at a rate similar to that of unexposed controls. Similar results were obtained in rabbits exposed to 173 ppm for 16 days or to 158 ppm for about 200 days. Slight growth depression was observed in male and female guinea pigs exposed to 158 ppm 1,4-DCB for 157 days, but only males showed a slight delay in growth when the exposure level was 341 ppm for 6 months. In male and female mice and in one female monkey, there were no effects on body weight after exposure to 1,4-DCB at air concentrations up to 158 ppm for as long as 7.1 months. In another intermediate-duration study, there were no effects on body weight gain in F344 rats and BDF_1 mice that were exposed to 25, 55, 120, 270, or 600 ppm of 1,4-DCB for 6 hours/day, 5 days/week for 13 weeks (Aiso et al. 2005a).

In a 2-generation study of the effects of inhalation exposure to 1,4-DCB in Sprague-Dawley rats, males and females were exposed to 0, 66.3, 211, or 538 ppm 1,4-DCB 6 hours/day for 10 weeks prior to mating. The females were also exposed during mating, and on Gd 0–19 and postnatal days 5–27; males were exposed throughout the study. Male F_0 body weight and body weight gain were significantly reduced in the 538 ppm group. Body weight gain was also significantly reduced in the 211 ppm group; however, the effect was seen at fewer observation periods. Female F_0 body weights were equivalent across all treatment groups during the entire prebreeding period. The F_1 generation males and females exposed to 538 ppm 1,4-DCB had lower body weights than did controls; however, these decreases were accompanied by decreased food consumption (Tyl and Neeper-Bradley 1989).

A chronic-duration inhalation study in male and female Wistar rats found that body weight was not significantly altered after exposure to 1,4-DCB at air concentrations up to 490–499 ppm, 5 hours/day, 5 days/week for 76 weeks (Riley et al. 1980a).

Other Systemic Effects.

1,2-Dichlorobenzene. No studies were located regarding other systemic effects in humans or animals following inhalation exposure to 1,2-DCB.

1,3-Dichlorobenzene. No studies were located regarding other systemic effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding other effects in humans following inhalation exposure to 1,4-DCB. Ascites, esophageal varices, hemorrhoids, and tarry stools are all secondary effects of subacute, yellow atrophy and cirrhosis of the liver (Cotter 1953).

A chronic-duration inhalation study in male and female Wistar rats found that food and water consumption was not significantly altered after exposure to 1,4-DCB at air concentrations up to 490–499 ppm, 5 hours/day, 5 days/week for 76 weeks (Riley et al. 1980a).

In a 2-generation study of the effects of inhalation exposure to 1,4-DCB in Sprague-Dawley rats, males and females were exposed to 0, 66.3, 211, or 538 ppm 1,4-DCB 6 hours daily for 10 weeks prior to mating. The females were also exposed during mating, and on Gd 0–19 and postnatal days 5–27; males were exposed throughout the study. Exposure of the F_0 and F_1 generations to 538 ppm 1,4-DCB resulted in clinical signs of toxicity such as decreased grooming, unkempt appearance, decreased food consumption, and dehydration (Tyl and Neeper-Bradley 1989).

3.2.1.3 Immunological and Lymphoreticular Effects

1,2-Dichlorobenzene. No studies were located regarding immunological effects in humans following inhalation exposure to 1,2-DCB.

No changes in absolute spleen weight or spleen histology were reported for rats (20/sex) or guinea pigs (8/sex) that were exposed to 93 ppm 1,2-DCB for 7 hours/day, 5 days/week for 6–7 months (Hollingsworth et al. 1958). Relative spleen weight was not determined. The scope of histological evaluations was not specifically reported; organs that were weighed appear to have been examined.

1,3-Dichlorobenzene. No studies were located regarding immunological effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. As mentioned in Section 3.2.1.2, dermal effects observed in a 69-year-old man who had been exposed to 1,4-DCB in his home for approximately 3 weeks (Nalbandian and Pearce 1965) may have been mediated by immunological mechanisms. In addition to petechiae, purpura, and swelling of his hands and feet, his serum showed a strong positive reaction to 1,4-DCB in an indirect basophil degranulation test. The authors stated that, to their knowledge, this was the first reported case of allergic (anaphylactoid) purpura induced by exposure to 1,4-DCB. Enlargement of the spleen was reported in a

woman who had been exposed to 1,4-DCB in her home for 3–4 months and in a man who used 1,4-DCB to preserve raw furs (Cotter 1953). This, however, was most likely a secondary response to hematological disturbances rather than an immunological effect.

A slight decrease in relative spleen weight was observed in male guinea pigs exposed to 1,4-DCB at an air concentration of 173 ppm, 7 hours/day, 5 days/week for 16 days (Hollingsworth et al. 1956); no effect was seen in rats under the same experimental conditions. In a chronic-duration inhalation study, groups of male and female Wistar rats exposed to 1,4-DCB 5 hours/day, 5 days/week for 76 weeks exhibited no gross or histopathological alterations in the cervical, thoracic, and mesenteric lymph nodes; spleen; or thymus at air concentrations up to 500 ppm (Riley et al. 1980a). No other immunological end points were evaluated.

No effects were found in an immunotoxicity study in which groups of 10 male SPF Hartley guinea pigs were exposed to 1,4-DCB by inhalation in concentrations of 0, 2, or 50 ppm for 12 weeks (schedule not specified) (Suzuki et al. 1991). The animals were sensitized with ovalbumin after 4 and 8 weeks of exposure to evaluate effects on antibody production. Determinations of serum IgE titers (passive cutaneous anaphylaxis test) and serum IgG and IgM titers (enzyme-linked immunosorbent assay) against ovalbumin, performed 1 and 2 weeks after the first sensitization and 1, 2, and 4 weeks after the second sensitization, showed no significant differences between the exposed and control groups. The passive cutaneous anaphylaxis test was also conducted with antiserum from the 50 ppm exposure group (collected 1 and 2 weeks after the first sensitization and 1, 2, and 4 weeks after the second sensitization) to determine if IgE antibodies were produced against 1,4-DCB; no antibodies against the compound were detected. Active systemic anaphylaxis was also evaluated in the 0 and 50 ppm exposure groups. An antigen mixture of 1,4-DCB and guinea pig serum albumin did not cause an anaphylactic reaction when intravenously injected in the animals 14 days after the last exposure. This study was reported in the Japanese literature; relevant information was obtained from the English abstract and data tables.

3.2.1.4 Neurological Effects

1,2-Dichlorobenzene. No studies were located regarding neurological effects in humans or animals following inhalation exposure to 1,2-DCB.

1,3-Dichlorobenzene. No studies were located regarding neurological effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. Information regarding neurological effects in humans exposed to 1,4-DCB via inhalation is limited to several case reports. A 60-year-old man whose home had been saturated with 1,4-DCB moth ball vapor for 3 or 4 months complained of persistent headache, numbness, clumsiness, and a burning sensation in his legs (consistent with peripheral nerve damage); he also showed slurred speech (Cotter 1953). In a more recent case study, a 25-year-old woman was exposed to high concentrations of 1,4-DCB from her bedroom, bedding, and clothing. She had used this compound liberally as an insect repellant for 6 years. The subject sought medical assistance because of severe ataxia, speech difficulties, and moderate weakness of her limbs. Brainstem auditory-evoked potentials (BAEPs) showed marked delays of specific brainwave patterns. Her symptoms gradually improved over the next 6 months after cessation of exposure and the BAEPs examined 8 months later had returned to normal. This study suggests that there may be measurable but reversible neurological effects associated with human inhalation exposure to 1,4-DCB (Miyai et al. 1988). The level of 1,4-DCB exposure was neither known nor estimated in either of the human case studies. In addition, there is no certainty that exposure to 1,4-DCB was the only factor associated with the toxic effects reported.

Neurological signs including marked tremors, weakness, and loss of consciousness were observed in rats, rabbits, and guinea pigs exposed to 798 ppm 1,4-DCB 8 hours/day, 5 days/week (Hollingsworth et al. 1956). In a chronic-duration study in rats, exposure to up to 500 ppm 1,4-DCB 5 hours/day, 5 days/week for 76 weeks did not cause gross or histological alterations in the brain, sciatic nerve, or spinal cord, but absolute brain weight was slightly decreased at the termination of the study (Riley et al. 1980a). Adult rats exposed 6 hours/day for 10 weeks to 538 ppm 1,4-DCB during a 2-generation study displayed symptoms associated with compound neurotoxicity, including tremors, ataxia, and hyperactivity (Tyl and Neeper-Bradley 1989). The animals also decreased their grooming behavior and developed an unkempt appearance. At sacrifice, the relative brain weights of the males, but not the females, were significantly increased compared to the controls.

3.2.1.5 Reproductive Effects

1,2-Dichlorobenzene. No studies were located regarding reproductive effects in humans or animals following inhalation exposure to 1,2-DCB.

A 2-generation inhalation reproduction study was conducted in which groups of Charles River CD (Sprague-Dawley derived) rats (30/sex/generation) were exposed to 1,2-DCB at levels of 0, 50, 150, or

394 ppm (Bio/dynamics 1989). F_0 adults were exposed for 6 hours/day, 7 days/week for a 10-week premating period and during mating. Following mating, F_0 males were exposed 6 hours/day, 7 days/week until sacrifice at 3–4 weeks postmating. Bred F_0 females were exposed for 6 hours/day on gestation days 0–19 and lactation days 5–28, then sacrificed postweaning. F_1 pups (29 days old) received similar exposures throughout an 11-week premating period, mating, gestation, and lactation. There were no exposure-related effects on reproductive performance or fertility indices in either generation.

No changes in absolute testicular weight or testicular histology were reported for male rats or guinea pigs that were exposed to 93 ppm 1,2-DCB for 7 hours/day, 5 days/week for 6–7 months (Hollingsworth et al. 1958). Relative testicular weight was not determined. The scope of histological evaluations in this study was not specifically reported; organs that were weighed also appear to have been examined.

1,3-Dichlorobenzene. No studies were located regarding reproductive effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding reproductive effects in humans after inhalation exposure to 1,4-DCB.

In an acute-duration study using pregnant Alderley-Park rats, whole-body exposure to 1,4-DCB at air concentrations up to 508.4 ppm, 6 hours/day from Gd 6 to 15 did not adversely affect the number of implantations, resorptions, viable fetuses, corpora lutea, or sex ratios (Hodge et al. 1977). A similar study in inseminated New Zealand White rabbits exposed whole-body to 1,4-DCB at air concentrations of 100, 300, or 800 ppm, 6 hours/day on Gd 6–18 found no differences between treated and control groups in the mean number of corpora lutea per dam, the mean number of implantation sites per dam, the mean number of resorptions per litter, or the number of totally resorbed litters. At 300 ppm, there was a significant increase ($p \leq 0.05$) in the percentage of resorbed implantations per litter and in the number of litters with resorptions; however, the results at 800 ppm were comparable to controls, and the percentage of litters with resorptions reported in the 300 ppm group was within the range reported for historical controls, suggesting this effect was not chemical- or dose-related (Hayes et al. 1985).

Exposure of rats and guinea pigs to 1,4-DCB at an air concentration of 173 ppm, 7 hours/day, 5 days/week for 2 weeks did not significantly alter relative testis weight. The same results were obtained after intermittently exposing rats and guinea pigs to 1,4-DCB at air concentrations up to 158 ppm for 5–7 months (Hollingsworth et al. 1956). There were no treatment-related effects on the reproductive organs

of male or female Wistar rats exposed to 1,4-DCB at concentrations up to 490–499 ppm, 5 hours/day, 5 days/week for 76 weeks (Riley et al. 1980a). The evaluation of reproductive end points included organ weights and histopathology.

In another chronic inhalation study, groups of 50 male and female F344/DuCrj rats and 50 male and 50 female Crj:BDF_1 mice were exposed to 1,4-DCB in concentrations of 0, 20, 75, or 300 ppm for 6 hours/day, 5 days/week for 104 weeks (Aiso et al. 2005b; Japan Bioassay Research Center 1995). Histological examinations included reproductive system tissues in both sexes (testis, epididymis, seminal vesicle, prostate, ovary, uterus, vagina, and mammary gland), but there were no exposure-related adverse findings in either species or sex (Aiso 2006).

The effects of 1,4-DCB vapors on the reproductive performance of Sprague-Dawley rats was assessed in a 2-generation study in which animals of both sexes were exposed before and during mating (Tyl and Neeper-Bradley 1989). The females were then exposed on Gd 0–19 and postnatal days 5–27. Effects on body weight, liver and kidney weight, and hepatocellular hypertrophy were found in the adult rats at exposure concentrations of 211 and 538 ppm and were indicative of toxicity to the breeding animals. These effects did not occur with the 66.3 ppm exposure concentration. Both generations of offspring exposed to the 538 ppm concentration had lower body weights than the controls at lactation day 4; average litter size and survival rates were decreased. When selected animals from the first filial generation were allowed to recover from the 1,4-DCB exposure for a 5-week period, body weights of the 538 ppm exposure group remained lower than those for the controls. The authors concluded that parental toxicity was the cause of the increased risk to offspring rather than inherent effects of 1,4-DCB on reproductive processes. In addition, no reduction in reproductive performance (as measured by the percentage of males successfully impregnating females) was observed in an inhalation study in which male mice were exposed to 1,4-DCB at 75–450 ppm for 6 hours/day for 5 days before being mated with virgin females (Anderson and Hodge 1976). These data are consistent with the data from the males used in the 2-generation study discussed above.

3.2.1.6 Developmental Effects

1,2-Dichlorobenzene. No studies were located regarding developmental effects in humans or animals following inhalation exposure to 1,2-DCB.

1,3-Dichlorobenzene. No studies were located regarding developmental effects in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding developmental effects in humans after inhalation exposure to 1,4-DCB.

Exposure of pregnant Alderley-Park rats to 1,4-DCB via inhalation at levels up to 508 ppm for 6 hours/day on Gd 6–15 did not result in developmental effects in the offspring (Hodge et al. 1977). End points examined included the number of viable fetuses, fetal weight, litter weight, sex ratio, external abnormalities, and skeletal and visceral abnormalities.

In a 2-generation study of the effects of inhalation exposure to 1,4-DCB in Sprague-Dawley rats, males and females that were exposed to 0, 66.3, 211, or 538 ppm 1,4-DCB 6 hours daily for 10 weeks prior to mating were assessed. The females were also exposed during mating, and on Gd 0–19 and postnatal days 5–27; males were exposed throughout the study. F_1 and F_2 pup body weights in the 538 ppm group were significantly reduced from postnatal day 0 to 28. The number of F_1 and F_2 pups that died during the perinatal period was significantly elevated in the 538 ppm group (Tyl and Neeper-Bradley 1989).

The developmental effects of 1,4-DCB have been evaluated in New Zealand White rabbits (Hayes et al. 1985). Pregnant rabbits were exposed to 1,4-DCB by inhalation at 800 ppm for 6 hours/day on Gd 6–18. At 300 ppm, there was a significant increase in the number of litters with resorptions and the percentages of resorbed implantations per litter; however, this effect was not seen at 800 ppm and was thus probably not treatment-related. An increased incidence of retroesophageal right subclavian artery present in the offspring was noted; it was not considered to constitute a teratogenic response to exposure to 1,4-DCB, but was considered only a minor variation.

3.2.1.7 Cancer

1,2-Dichlorobenzene. No studies were located regarding cancer in humans or animals following inhalation exposure to 1,2-DCB.

1,3-Dichlorobenzene. No studies were located regarding cancer in humans or animals following inhalation exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding cancer in humans after inhalation exposure to 1,4-DCB.

No evidence of carcinogenicity was observed in a long-term inhalation study in rats that were exposed to 1,4-DCB at 75 or 500 ppm intermittently for 76 weeks (Riley et al. 1980a). The reported lack of extensive organ toxicity in this study (compared with results seen in oral studies described in Section 3.2.2.2) strongly suggests that a maximum tolerated dose (MTD) was not achieved. In addition, a less-than-lifetime dosing regimen was used. The experimental design limitations preclude reliable evaluation of potential inhalation carcinogenicity based on this study.

The carcinogenicity of 1,4-DCB was more recently evaluated in groups of 50 male and female F344/DuCrj rats, and 50 male and 50 female Crj:BDF_1 mice, following exposure to concentrations of 0, 20, 75, or 300 ppm for 6 hours/day, 5 days/week for 104 weeks (Aiso et al. 2005b; Japan Bioassay Research Center 1995). Comprehensive histological evaluations (including nasal cavity, trachea, and lungs) showed no compound-related neoplastic changes in rats, although incidences of liver and lung tumors were elevated in mice. The liver tumors were induced in mice of both sexes, generally increased only at 300 ppm, and were comprised of several tumor types. Liver tumors reported to be significantly increased ($p \leq 0.05$, Fisher's Exact test) in male mice were hepatocellular carcinoma (12/49, 17/49, 16/50, 38/49; $p \leq 0.01$ at high dose), hepatoblastoma (0/49, 2/49, 0/50, 8/49; $p \leq 0.01$ at high dose) and hepatic histiocytic sarcoma (0/49, 3/49, 1/50, 6/49; $p \leq 0.05$ at high dose). Liver tumors reported to be significantly increased in female mice were hepatocellular carcinoma (2/50, 4/50, 2/49, 41/50; $p \leq 0.01$ at high dose), hepatocellular adenoma (2/50, 10/50, 6/49, 20/50; $p \leq 0.05$ at low and high doses), hepatocellular carcinoma or adenoma (4/50, 13/50, 7/49, 45/50; $p \leq 0.05$ at low and high doses), and hepatoblastoma (0/50, 0/50, 0/49, 6/50; $p \leq 0.05$ at high dose). Although the hepatocellular adenomas were increased in female mice at 20 and 300 ppm, the relevance of the increase at 20 ppm is unclear given the lack of significant change at 75 ppm. Lung bronchoalveolar adenoma and carcinoma were significantly increased in female mice (1/50, 4/50, 2/49, 7/50; $p \leq 0.05$ at high dose). Except for hepatoblastoma, all of the aforementioned liver and lung tumor incidences were reported to have a significant positive linear trend by the Peto test and/or Cochran-Armitage test.

3.2.2 Oral Exposure

Most of the data described in this section were derived from laboratory studies in which 1,2-, 1,3-, and 1,4-DCB were administered to test animals via gavage. In addition, two human case studies of 1,4-DCB

consumption are described. Case studies are not generally scientifically equivalent to well-conducted epidemiologic studies or laboratory experiments and should be viewed only as providing contributory evidence that 1,4-DCB may have caused the reported effects. The available case studies do not provide unequivocal proof that 1,4-DCB is solely responsible for the reported toxicological effects in humans. The highest NOAEL and all reliable LOAEL values after oral exposure to 1,2-, 1,3-, and 1,4-DCB are recorded in Tables 3-3, 3-4, and 3-5, respectively, and plotted in Figures 3-3, 3-4, and 3-5, respectively.

3.2.2.1 Death

1,2-Dichlorobenzene. No studies were located regarding death in humans after oral exposure to 1,2-DCB.

Single-dose LD_{50} values of 500 and 1,516 mg/kg have been reported for 1,2-DCB in rats administered the compound in oil by gavage (Ben-Dyke et al. 1970; Monsanto 1989). Rats that were gavaged with a 25% solution of 1,2-DCB in peanut oil at a dose of 675 mg/kg/day for 3 days were considered unlikely to survive further exposures (DuPont 1982). Guinea pigs that were treated with a single gavage dose of 1,2-DCB as a 50% solution in olive oil had no deaths at 800 mg/kg and 100% mortality at 2,000 mg/kg (Hollingsworth et al. 1958).

Rats that were administered 1,2-DCB in oil by gavage for 14 consecutive days and observed until day 20 experienced 100% mortality at 1,000 mg/kg/day and no deaths at 500 mg/kg/day and lower doses (NTP 1985). Mice that were similarly treated with 1,2-DCB for 14 days had 80% mortality in both sexes at 250 mg/kg/day (lowest tested dose) and 80–100% mortality at ≥500 mg/kg/day (NTP 1985). The reliability of the 14-day findings is uncertain because there were no clear effects of gavage exposure to 1,2-DCB in oil on survival in rats or mice exposed to ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985), rats exposed to 400 mg/kg/day on 7 days/week for 90 days (Robinson et al. 1991), or rats or mice exposed to ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985). Information in the longer-term NTP (1985) studies suggests that gavage error might have contributed to some of the deaths in the 14-day studies.

1,3-Dichlorobenzene. No studies were located regarding death in humans after oral exposure to 1,3-DCB.

Table 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
ACUTE EXPOSURE									
Death									
1	Rat (NS)	once (NS)				500	(LD50)	Ben-Dyke et al. 1970 1,2-dichlorobenzene	
2	Rat (NS)	once (GO)				1500	(lowest lethal dose)	DuPont 1982 1,2-dichlorobenzene	
3	Rat (NS)	3 d 1 x/d (GO)				675	(unlikely to survive further exposure)	DuPont 1982 1,2-dichlorobenzene	
4	Rat (NS)	once (G)				1516	(LD50)	Monsanto 1989 1,2-dichlorobenzene	
5	Rat (Fischer- 344)	14 d 7 d/wk 1 x/d (GO)				1000	(100% mortality)	NTP 1985 1,2-dichlorobenzene	
6	Mouse (B6C3F1)	14 d 7 d/wk 1 x/d (GO)				250	(80% mortality)	NTP 1985 1,2-dichlorobenzene	
7	Gn Pig (NS)	once (GO)				2000	(100% mortality)	Hollingsworth et al. 1958 1,2-dichlorobenzene	
Systemic									
8	Rat (NS)	once (GO)	Hepatic			1500	(central necrosis)	DuPont 1982 1,2-dichlorobenzene	
			Renal			1500	(albuminous fluid and casts in tubules)		

Table 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
9	Rat (NS)	3 d 1 x/d (GO)	Bd Wt			675 (10% body weight loss)	DuPont 1982 1,2-dichlorobenzene	
10	Rat (Fischer- 344)	14 d 7 d/wk 1 x/d (GO)	Hepatic	1000			NTP 1985 1,2-dichlorobenzene	
			Bd Wt	500[b] M 1000 F	1000 M (12% reduced body weight gain)			

Table 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
11	Rat (Sprague-Dawley)	10 d 7 d/wk 1 x/d (GO)	Resp	300 M			Robinson et al. 1991 1,2-dichlorobenzene	
			Cardio	300				
			Gastro	300 M				
			Hemato	300 M				
			Musc/skel	300 M				
			Hepatic	150 M 75[c] F	300 M (slight necrosis, increased serum ALT) 150[b] F (increased liver weight)			
			Renal	300 M				
			Endocr	300 M				
			Dermal	300				
			Bd Wt	150[b] M 300 F	300 M (10.9% reduced body weight gain)			
12	Mouse (B6C3F1)	14 d 7 d/wk 1 x/d (GO)	Hepatic		250 (hepatocellular degeneration)		NTP 1985 1,2-dichlorobenzene	

Table 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
13	Mouse (B6C3F1)	14 d 7 d/wk 1 x/d (GO)	Hepatic		500 (hepatocellular necrosis and degeneration)		NTP 1985 1,2-dichlorobenzene	
			Bd Wt	500				
Immuno/ Lymphoret								
14	Rat (Sprague-Dawley)	10 d 7 d/wk 1 x/d (GO)		300			Robinson et al. 1991 1,2-dichlorobenzene	
Developmental								
15	Rat (Sprague-Dawley)	10 d Gd 6-15 (G)		200 F			Ruddick et al. 1983 1,2-dichlorobenzene	
INTERMEDIATE EXPOSURE								
Systemic								
16	Rat (NS)	192 d 5 d/wk	Hemato	376 F			Hollingsworth et al. 1958 1,2-dichlorobenzene	
			Hepatic		376 F (slight to moderate cloudy swelling)			
			Renal	376 F				
			Bd Wt	376 F				

Table 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
17	Rat (Fischer- 344)	13 wk 5 d/wk 1 x/d (GO)	Resp	500			NTP 1985 1,2-dichlorobenzene	
			Cardio	500				
			Gastro	500				
			Hemato	500				
			Musc/skel	500				
			Hepatic	60[d]	125 (increased liver weight)			
			Renal	250[b] M 500 F	500 M (renal tubular degeneration)			
			Endocr	500				
			Dermal	500				
			Ocular	500				
			Bd Wt	250[b] M 500 F	500 M			
18	Rat (albino)	15 d 1 x/d (G)	Hepatic			455 M (necrosis and fatty changes, porphyria)	Rimington and Ziegler 1963 1,2-dichlorobenzene	

Table 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
19	Rat (Sprague-Dawley)	90 d 7 d/wk 1 x/d (GO)	Resp	400 M			Robinson et al. 1991 1,2-dichlorobenzene	
			Cardio	400				
			Hepatic		400 (centrilobular degeneration, single cell necrosis)			
			Renal	400				
			Endocr	400 M				
			Bd Wt		400 M (12.8% decreased body weight gain)			

Table 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
20	Mouse (B6C3F1)	13 wk 5 d/wk 1 x/d (GO)	Resp	500			NTP 1985 1,2-dichlorobenzene	
			Cardio	250	500 (mineralization of myocardial fibers)			
			Gastro	500				
			Hemato	500				
			Musc/skel	250	500 (mineralization of myocardial and skeletal muscle fibers)			
			Hepatic	125[b] M 250 F	250[b] M (single cell necrosis, hepatocellular degeneration)			
					500 F			
			Renal	500				
			Endocr	500				
			Dermal	500				
			Ocular	500				
			Bd Wt	500	500 (11-19% reduced body weight gain)			

Table 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
21	Rat (Fischer- 344)	13 wk 5 d/wk 1 x/d (GO)		250 M	500 M (lymphoid depletion in thymus)		NTP 1985 1,2-dichlorobenzene	
22	Rat (Sprague-Dawley)	90 d 7 d/wk 1 x/d (GO)		400			Robinson et al. 1991 1,2-dichlorobenzene	
23	Mouse (B6C3F1)	13 wk 5 d/wk 1 x/d (GO)		500			NTP 1985 1,2-dichlorobenzene	
Neurological								
24	Rat (Fischer- 344)	13 wk 5 d/wk 1 x/d (GO)		500			NTP 1985 1,2-dichlorobenzene	
25	Rat (albino)	15 d 1 x/d (G)				455 M (ataxia, clonic contractions)	Rimington and Ziegler 1963 1,2-dichlorobenzene	
26	Rat (Sprague-Dawley)	90 d 7 d/wk 1 x/d (GO)		400			Robinson et al. 1991 1,2-dichlorobenzene	
27	Mouse (B6C3F1)	13 wk 5 d/wk 1 x/d (GO)		500			NTP 1985 1,2-dichlorobenzene	

Table 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
28	Rat (Fischer- 344)	13 wk 5 d/wk 1 x/d (GO)		500			NTP 1985 1,2-dichlorobenzene	
29	Mouse (B6C3F1)	13 wk 5 d/wk 1 x/d (GO)		500			NTP 1985 1,2-dichlorobenzene	
CHRONIC EXPOSURE								
Systemic								
30	Rat (Fischer- 344)	103 wk 5 d/wk 1 x/d (GO)	Resp	120			NTP 1985 1,2-dichlorobenzene	
			Cardio	120				
			Gastro	120				
			Musc/skel	120				
			Hepatic	120				
			Renal	120				
			Endocr	120				
			Dermal	120				
			Ocular	120				
			Bd Wt	120				

Table 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
31	Mouse (B6C3F1)	103 wk 5 d/wk 1 x/d (GO)	Resp	120			NTP 1985 1,2-dichlorobenzene	
			Cardio	120				
			Gastro	120				
			Musc/skel	120				
			Hepatic	120				
			Renal	60[e]	120 (renal tubular regeneration)			
			Endocr	120				
			Dermal	120				
			Ocular	120				
			Bd Wt	120				
Immuno/ Lymphoret								
32	Rat (Fischer- 344)	103 wk 5 d/wk 1 x/d (GO)		120			NTP 1985 1,2-dichlorobenzene	
33	Mouse (B6C3F1)	103 wk 5 d/wk 1 x/d (GO)		120			NTP 1985 1,2-dichlorobenzene	

Table 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Neurological								
34	Rat (Fischer- 344)	103 wk 5 d/wk 1 x/d (GO)		120			NTP 1985 1,2-dichlorobenzene	
35	Mouse (B6C3F1)	103 wk 5 d/wk 1 x/d (GO)		120			NTP 1985 1,2-dichlorobenzene	
Reproductive								
36	Rat (Fischer- 344)	103 wk 5 d/wk 1 x/d (GO)		120			NTP 1985 1,2-dichlorobenzene	

a The number corresponds to entries in Figure 3-3.

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

c Study result used to derive an acute-duration oral Minimal Risk Level (MRL) of 0.7 mg/kg/day for 1,2-DCB, as described in detail in Appendix A. Benchmark dose analysis was performed on liver weight data to select a point of departure, which was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

d Study result used to derive an intermediate-duration oral Minimal Risk Level (MRL) of 0.6 mg/kg/day for 1,2-DCB, as described in detail in Appendix A. Benchmark dose analysis was performed on liver weight data to select a point of departure, which was adjusted for daily exposure, then divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

e Study result used to derive a chronic-duration oral Minimal Risk Level (MRL) of 0.3 mg/kg/day for 1,2-DCB, as described in detail in Appendix A. Benchmark dose analysis was performed on incidences of kidney lesions to select a point of departure, which was adjusted for daily exposure, then divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

ALT = alamine aminotransferase; Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; Gn pig = guinea pig; (GO) = gavage in oil; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; Resp = respiratory; x = time(s)

Figure 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral

Acute (≤14 days)

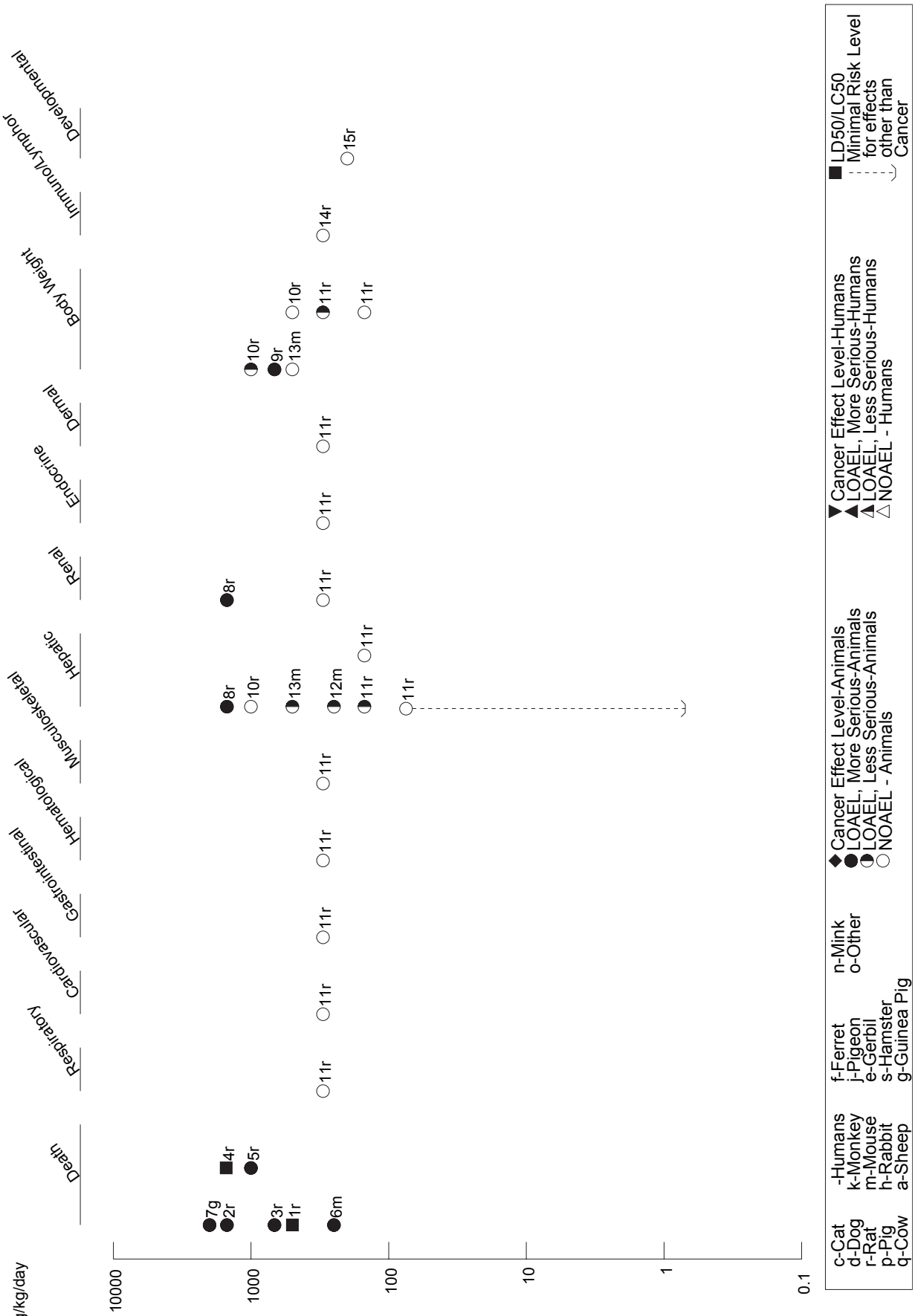


Figure 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

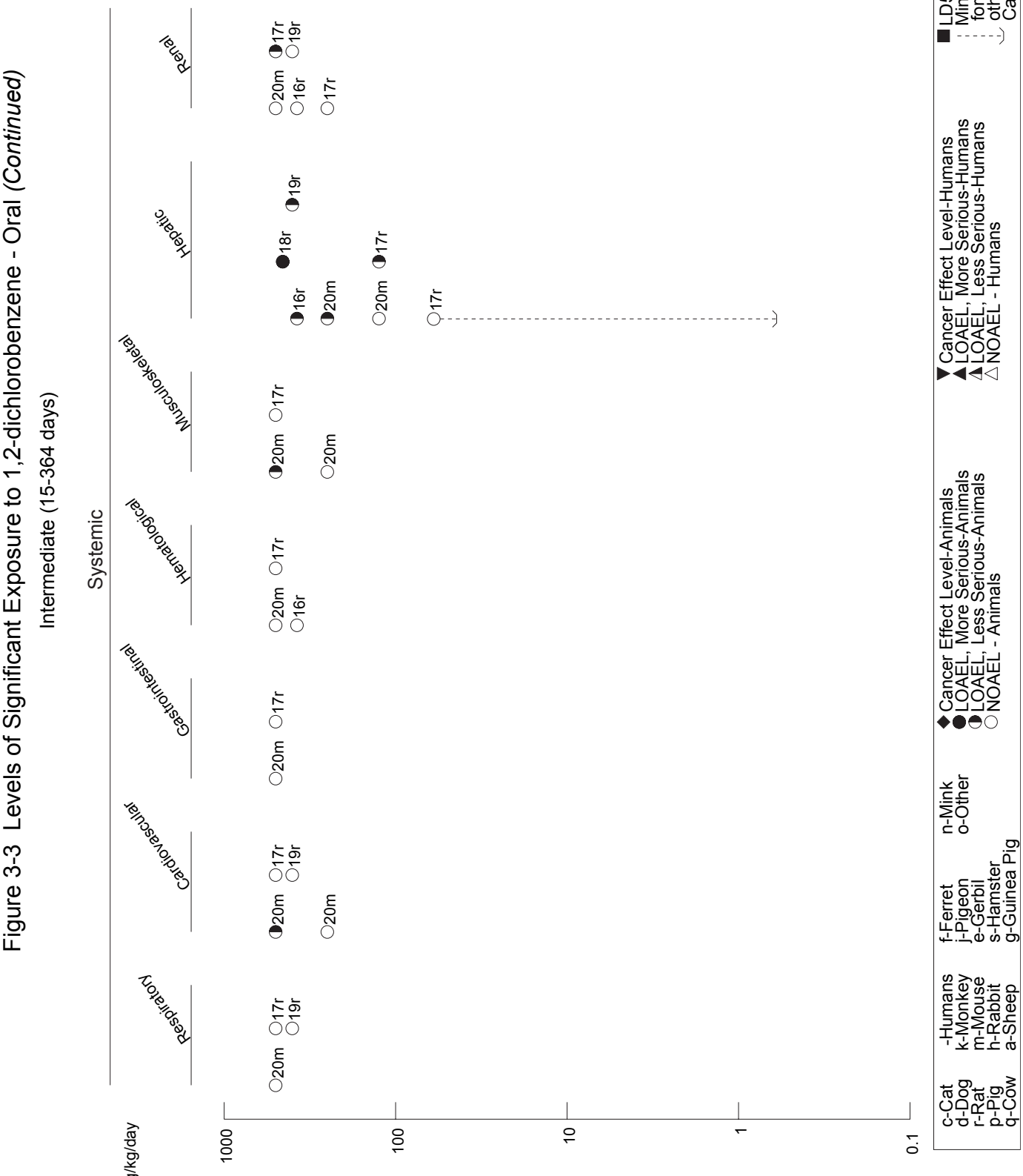


Figure 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

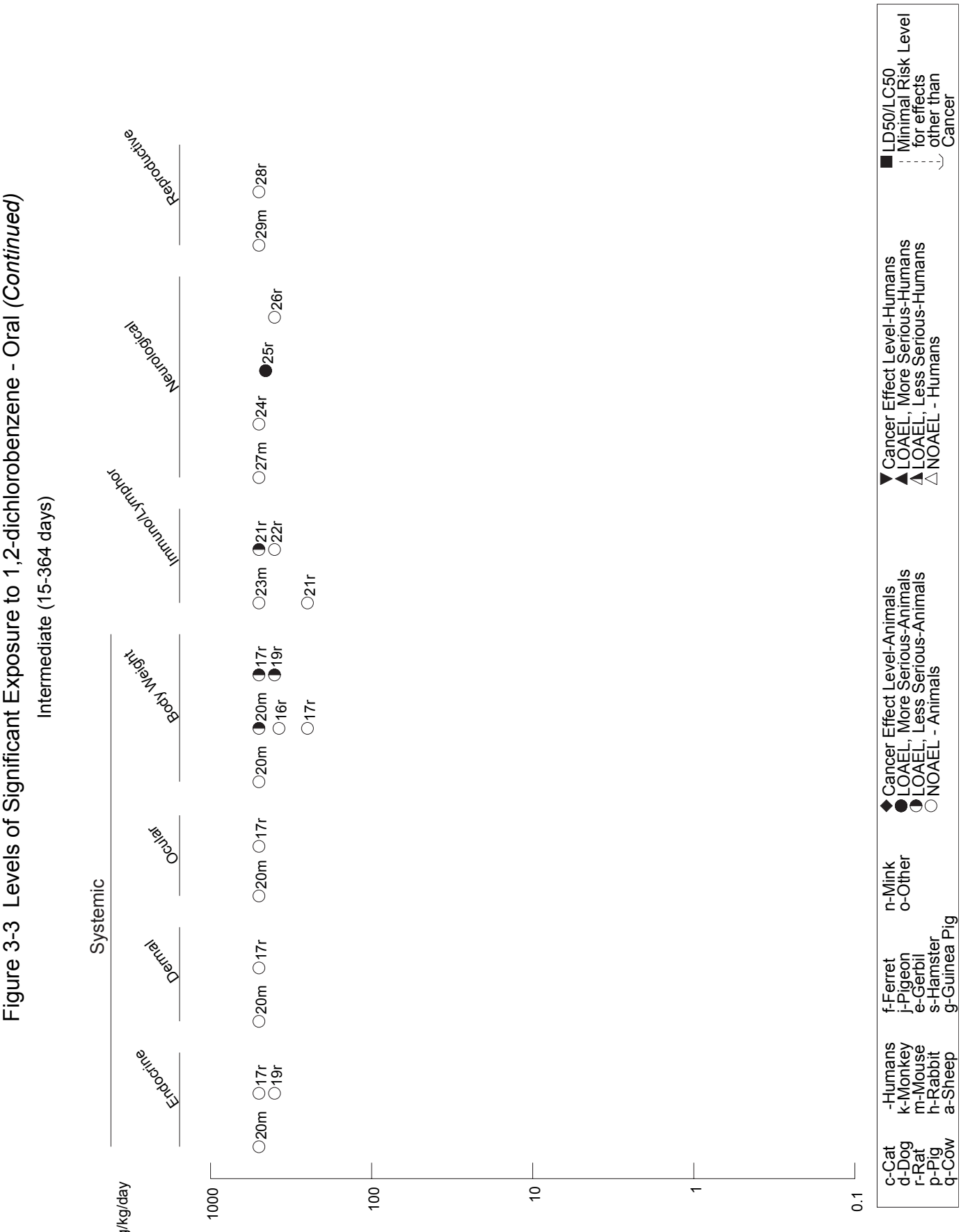


Figure 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral *(Continued)*

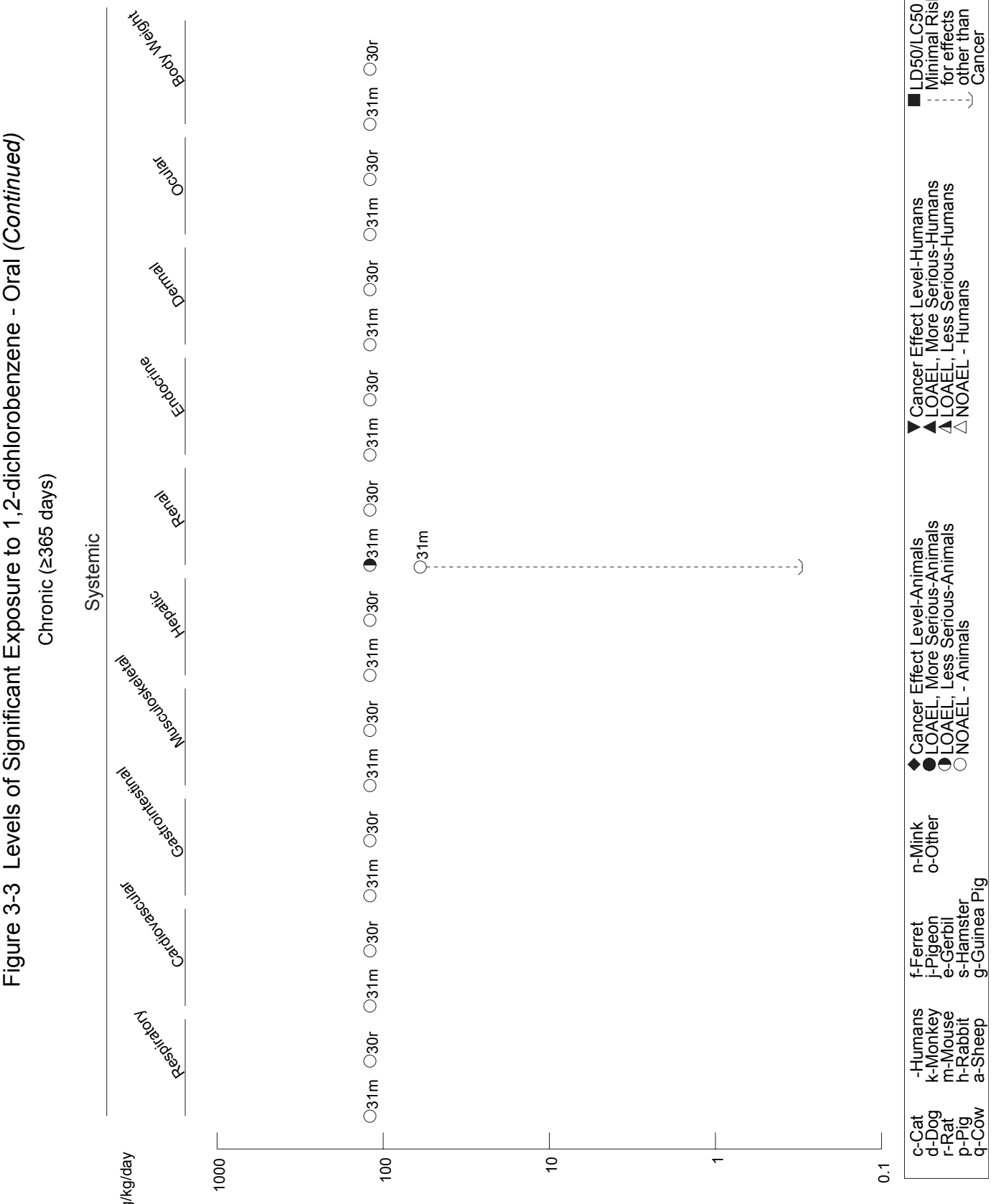


Figure 3-3 Levels of Significant Exposure to 1,2-dichlorobenzene - Oral *(Continued)*

Chronic (≥365 days)

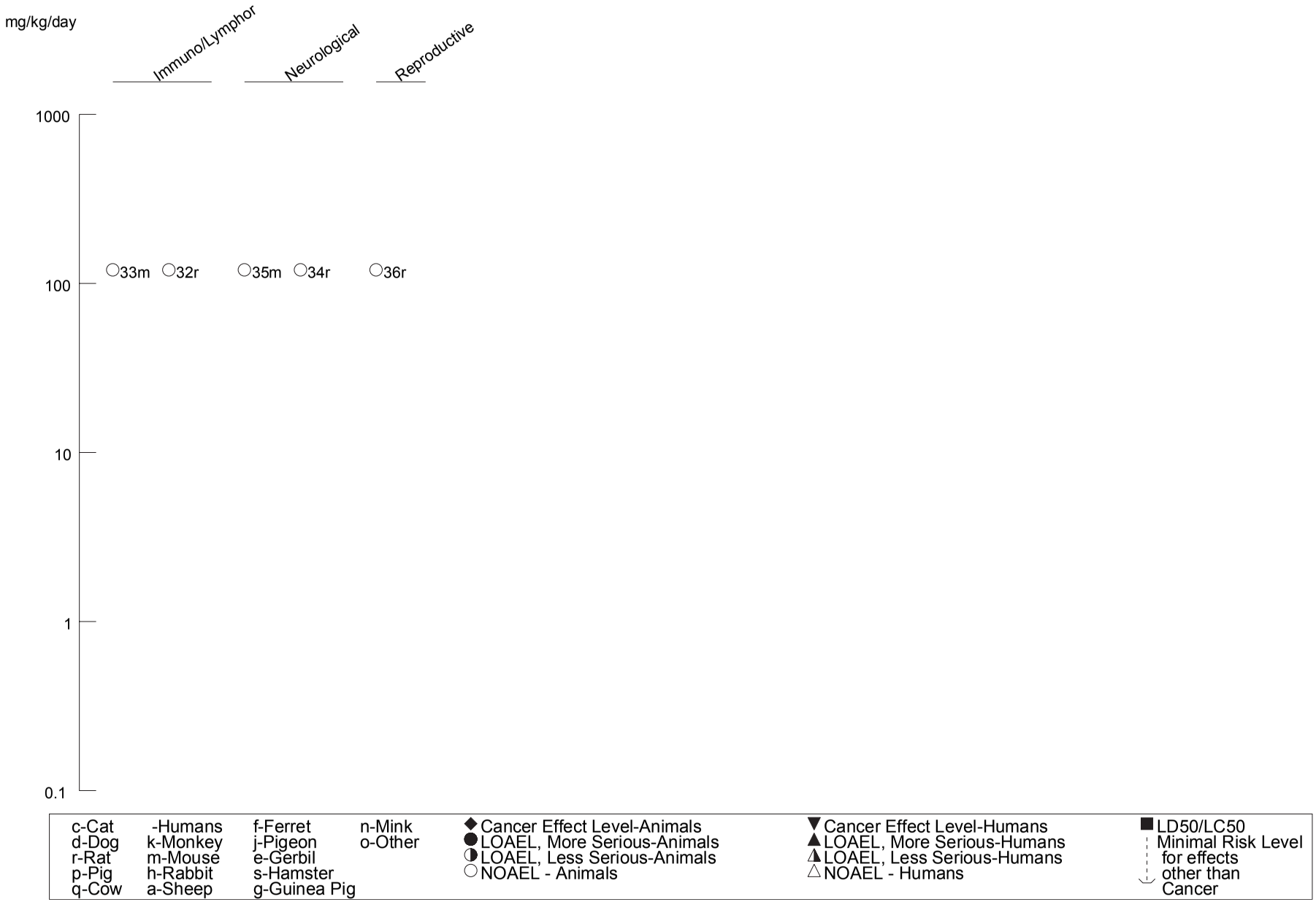


Table 3-4 Levels of Significant Exposure to 1,3-dichlorobenzene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	once (G)				1200 M (14-day LD50) 1000[b] F (14-day LD50)	Monsanto 1980 1,3-dichlorobenzene	
Systemic								
2	Rat (Sprague-Dawley)	10 d 7 d/wk (GO)	Resp	735			McCauley et al. 1995 1,3-dichlorobenzene	
			Gastro	735				
			Hemato	735				
			Musc/skel	735				
			Hepatic	37[c] M 147 F	368	(increased liver weight, cytoplasmic vacuolization)		
					147	(increased liver weight)		
			Renal	735				
			Endocr	735				
			Dermal	735				
			Bd Wt	368	735	(reduced body weight gain)		
Immuno/ Lymphoret								
3	Rat (Sprague-Dawley)	10 d 7 d/wk (GO)		735			McCauley et al. 1995 1,3-dichlorobenzene	
Neurological								
4	Rat (Sprague-Dawley)	10 d 7 d/wk (GO)		735			McCauley et al. 1995 1,3-dichlorobenzene	

Table 3-4 Levels of Significant Exposure to 1,3-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
5	Rat (Sprague-Dawley)	10 d 7 d/wk (GO)		735			McCauley et al. 1995 1,3-dichlorobenzene	
Developmental								
6	Rat (Sprague-Dawley)	10 d Gd 6-15 (G)		200 F			Ruddick et al. 1983 1,3-dichlorobenzene	

Table 3-4 Levels of Significant Exposure to 1,3-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE								
Systemic								
7	Rat (Sprague-Dawley)	90 d 7 d/wk (GO)	Resp	588			McCauley et al. 1995 1,3-dichlorobenzene	
			Gastro	588				
			Hemato	37[b] M 147 F	147[b] M (increased leukocyte levels)			
					588 F (increased leukocyte levels)			
			Musc/skel	588				
			Hepatic		9[b] M (increased serum AST and cholesterol levels)			
					37 F (increased serum AST and cholesterol levels)			
			Renal	588				
			Endocr	9 F	9 M (reduced colloidal density in thyroid follicles)			
					147[d] M (increased cytoplasmic vacuolization in pituitary pars distalis)			
					37 F (reduced colloidal density in thyroid follicles)			
			Dermal	588				
			Bd Wt	147	588 (body weight gain was reduced 24% in males and 10% in females)			

Table 3-4 Levels of Significant Exposure to 1,3-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
8	Rat (Sprague-Dawley)	90 d 7 d/wk (GO)		588			McCauley et al. 1995 1,3-dichlorobenzene	
Neurological								
9	Rat (Sprague-Dawley)	90 d 7 d/wk (GO)		588			McCauley et al. 1995 1,3-dichlorobenzene	

a The number corresponds to entries in Figure 3-4

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-4. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c Study result used to derive an acute-duration oral Minimal Risk Level (MRL) of 0.4 mg/kg/day for 1,3-DCB, as described in detail in Appendix A. Benchmark dose analysis was performed on liver weight data to select a point of departure, which was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

d Study result used to derive an intermediate-duration oral Minimal Risk Level (MRL) of 0.02 mg/kg/day for 1,3-DCB, as described in detail in Appendix A. Benchmark dose analysis was performed on incidences of pituitary lesions to select a point of departure, which was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

AST = aspartate aminotransferase; Bd Wt = body weight; d = day(s); Endocr = endocrine; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; Gn pig = guinea pig; (GO) = gavage in oil; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; occup = occupational; ppm = parts per million; Resp = respiratory; wk = week(s)

Figure 3-4 Levels of Significant Exposure to 1,3-dichlorobenzene - Oral

Acute (≤14 days)

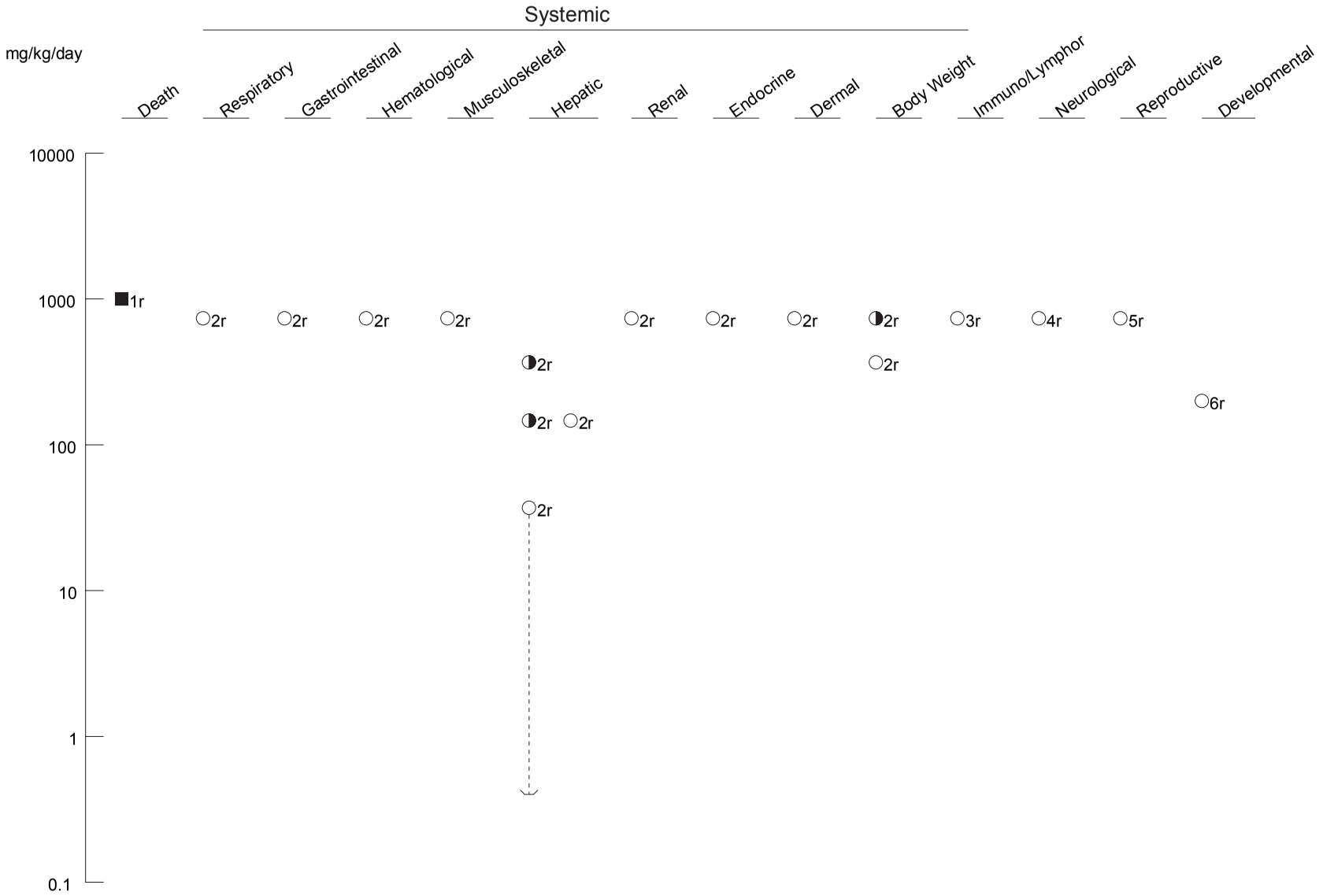


Figure 3-4 Levels of Significant Exposure to 1,3-dichlorobenzene - Oral *(Continued)*

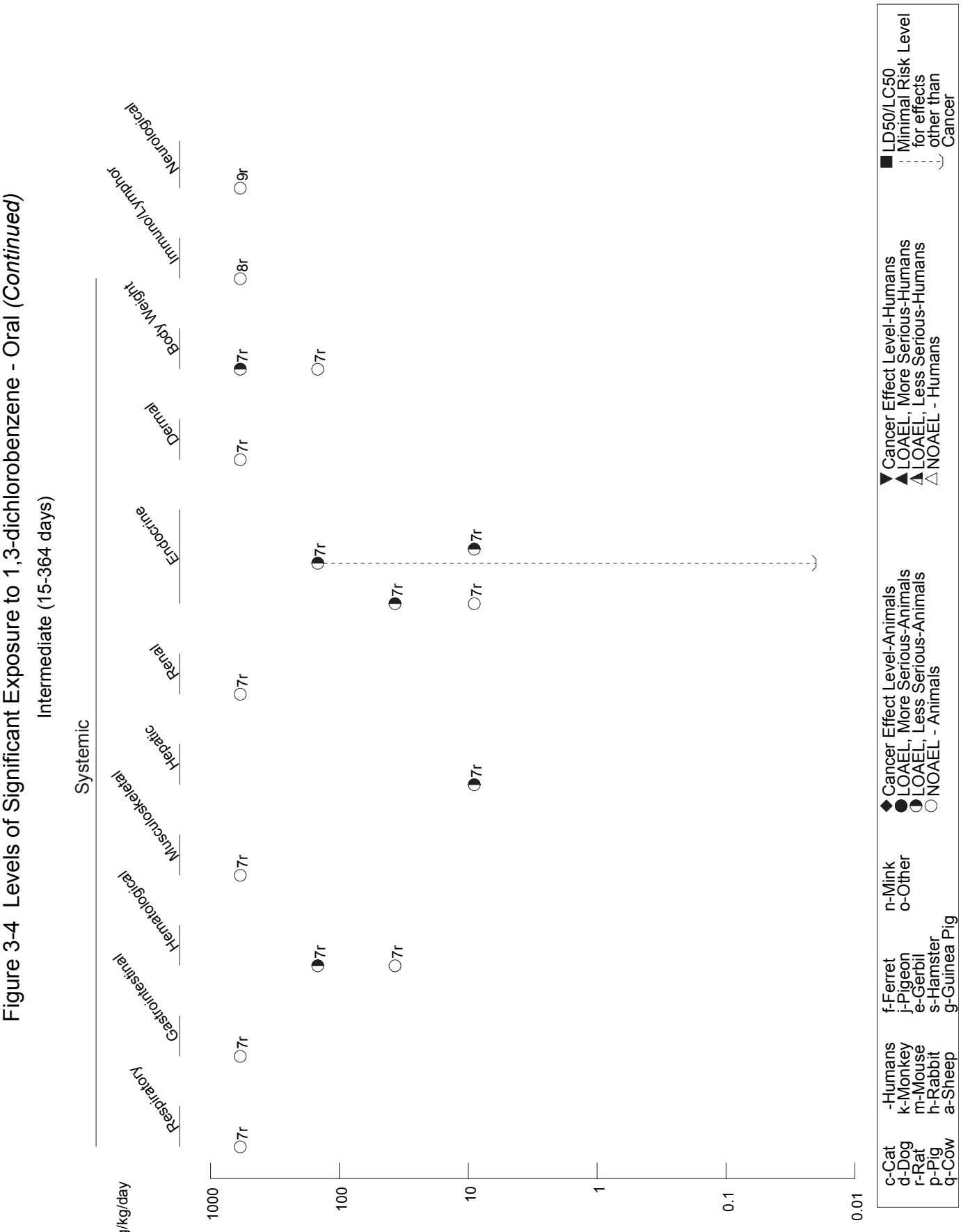


Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sherman)	once (GO)				3863 M (LD50) 3790[b] F (LD50)	Gaines and Linder 1986 1,4-dichlorobenzene	
2	Rat (NS)	once (GO)				4000 (LD100)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
3	Rat (Fischer- 344)	14 d 1 x/d (GO)				2000 M (5/5 males died) 1000[b] F (4/5 females died)	NTP 1987 1,4-dichlorobenzene	
4	Mouse (B6C3F1)	14 d 1 x/d (GO)				4000 (10/10 deaths by day 4)	NTP 1987 1,4-dichlorobenzene	
5	Gn Pig (NS)	once (GO)				2800 (LD100)	Hollingsworth et al. 1956 1,4-dichlorobenzene	
Systemic								
6	Rat (Fischer- 344)	once (GO)	Hemato	2790 M			Allis et al. 1992 1,4-dichlorobenzene	
			Hepatic		95 M (decreased relative liver weight)	475 M (centrilobular vacuolar degeneration)		
7	Rat (Wistar)	3 d 1 x/d (G)	Hepatic	250 F			Ariyoshi et al. 1975 1,4-dichlorobenzene	
			Bd Wt	250 F				

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
8	Rat (albino)	14 d 1 x/d (GO)	Hepatic	10 M	20 M (increase in glucuronyl transferase and EPN detoxification activities)		Carlson and Tardiff 1976 1,4-dichlorobenzene	
9	Rat (albino)	14 d 1 x/d (GO)	Hepatic	300 M	650 M (increased serum isocitrate dehydrogenase)		Carlson and Tardiff 1976 1,4-dichlorobenzene	
10	Rat (albino)	14 d 1 x/d (GO)	Hepatic		650 M (decreased hexobarbital sleeping time; increased serum isocitrate dehydrogenase)		Carlson and Tardiff 1976 1,4-dichlorobenzene	
11	Rat (Fischer- 344)	once (GO)	Renal	500 F	500 M (protein droplet formation)		Charbonneau et al. 1987 1,4-dichlorobenzene	
12	Rat (Fischer- 344)	7 d 1 x/d (GO)	Renal		120 M (protein droplet formation)		Charbonneau et al. 1987 1,4-dichlorobenzene	
13	Rat (Fischer- 344)	once (GO)	Hepatic		600 F (increased liver weight)		Eldridge et al. 1992 1,4-dichlorobenzene	
			Bd Wt	600 F				

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
14	Rat (Fischer- 344)	once (GO)	Hepatic		600 F (centrilobular hepatocyte vacuolation)		Eldridge et al. 1992 1,4-dichlorobenzene	
15	Rat (Fischer- 344)	1 wk 5 d/wk 1 x/d (GO)	Hepatic	25 M	75 M (increased microsomal 7-pentaoxyresorufin O - dependentylase activity)		Lake et al. 1997 1,4-dichlorobenzene	
			Renal	300 M				
			Bd Wt	150 M	300 M (approximately 10% decreased body weight gain)			
16	Rat (Fischer- 344)	14 d 1 x/d (GO)	Bd Wt	500[b] M 1000 F	1000 M (7-12% decrease in final body weight)		NTP 1987	
17	Rat (Fischer- 344)	14 d 1 x/d (GO)	Bd Wt	500	1000 (13.5% reduction in final body weight in males, 16.7% in females)		NTP 1987 1,4-dichlorobenzene	
18	Rat (albino)	5 d 1 x/d (G)	Hepatic			850 M (porphyria; degeneration of hepatocytes; focal necrosis)	Rimington and Ziegler 1963 1,4-dichlorobenzene	

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
19	Rat (albino)	5 d 1 x/d (G)	Hepatic			770 M (porphyria; degeneration of hepatocytes; focal necrosis)	Rimington and Ziegler 1963 1,4-dichlorobenzene	
			Bd Wt	770 M				
			Other		770 M (loss of appetite)			
20	Mouse (B6C3F1)	once (GO)	Hepatic		600 (increased liver weight)		Eldridge et al. 1992 1,4-dichlorobenzene	
			Bd Wt	600				
21	Mouse (B6C3F1)	once (GO)	Hepatic		600 (centrilobular hepatocyte vacuolation)		Eldridge et al. 1992 1,4-dichlorobenzene	
22	Mouse (B6C3F1)	1 wk 5 d/wk 1 x/d (GO)	Hepatic		300 M (increased relative liver weight)		Lake et al. 1997 1,4-dichlorobenzene	
			Renal	600 M				
			Bd Wt	600 M				
23	Mouse (B6C3F1)	14 d 1 x/d (GO)	Bd Wt	1000			NTP 1987 1,4-dichlorobenzene	

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
24	Mouse (B6C3F1)	14 d 1 x/d (GO)	Bd Wt		250 M (13.3% reduction in final body weight)		NTP 1987 1,4-dichlorobenzene	
25	Mouse (B6C3F1)	4 d 1 x/d (GO)	Hepatic		300 (increased liver weight and hepatocyte proliferation)		Umemura et al. 1992 1,4-dichlorobenzene	
			Renal	600				
26	Mouse (B6C3F1)	once	Hepatic	1000 M	1800 M (increased ALT activity; severe centrilobular hepatocyte swelling)		Umemura et al. 1996 1,4-dichlorobenzene	
27	Mouse (B6C3F1)	once	Hepatic		1800 M (increased ALT activity; increased BrdU labeling)		Umemura et al. 1996 1,4-dichlorobenzene	
Neurological								
28	Rat (albino)	5 d 1 x/d (G)				770 M (clonic contractions; slight tremors; hemiparesis)	Rimington and Ziegler 1963 1,4-dichlorobenzene	
Reproductive								
29	Rat (CD)	10 d Gd 6-15 1 x/d (GO)		1000 F			Giavini et al. 1986 1,4-dichlorobenzene	

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Developmental								
30	Rat (CD)	10 d Gd 6-15 1 x/d (GO)		250 F	500 F (increased incidence of fetuses with an extra rib)		Giavini et al. 1986 1,4-dichlorobenzene	
INTERMEDIATE EXPOSURE								
Death								
31	Rat (Fischer- 344)	13 wk 5 d/wk (GO)				1200[b] M (5/10 died) 1500 F (9/10 died)	NTP 1987 1,4-dichlorobenzene	
32	Mouse (B6C3F1)	13 wk 5 d/wk (GO)				1500 (3/10 males and 5/10 females died)	NTP 1987 1,4-dichlorobenzene	
Systemic								
33	Rat (NS)	30-120 d 1 x/d (GO)	Hepatic	200 F			Carlson 1977 1,4-dichlorobenzene	
34	Rat (Fischer- 344)	13 wk 5 d/wk (GO)	Hepatic		600 F (increased liver weight; hypertrophic centrilobular hepatocytes)		Eldridge et al. 1992 1,4-dichlorobenzene	
			Bd Wt	600 F				

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
35	Rat (NS)	192 d 5 d/wk (GO)	Hemato	188 F			Hollingsworth et al. 1956 1,4-dichlorobenzene	
			Hepatic		188 F (slight increase in liver weight; not quantified)	376 F (slight cirrhosis, focal necrosis)		
			Renal		188 F (slight increase in kidney weight; not quantified)			
			Ocular	376 F				
36	Rat (Fischer- 344)	4 or 13 wk 5 d/wk 1 x/d (GO)	Hepatic	25 M	75 M (increased relative liver weight, induction of microsomal P450 and 7-pentoxyresorufin O-depentylase activity)		Lake et al. 1997 1,4-dichlorobenzene	
			Renal	75 M	150 M (increased relative kidney weight)			
			Bd Wt	75 M	150 M (approximately 10% decreased body weight gain)			
37	Rat (Wistar)	42 d Gd 1- pnd 21 (F)	Bd Wt	2 F			Makita et al. 2004, 2005 1,4-dichlorobenzene	

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
38	Rat (Fischer- 344)	13 wk 5 d/wk (GO)	Resp	600			NTP 1987 1,4-dichlorobenzene	
			Cardio	600				
			Gastro	600				
			Musc/skel	600				
			Hepatic	600				
			Renal	300[b] M	600 M (tubular degeneration)			
				600 F				
			Endocr	600				
			Dermal	600				
			Ocular	600				
			Bd Wt	600				

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
39	Rat (Fischer- 344)	13 wk 5 d/wk (GO)	Resp	900	1200 (epithelial necrosis of nasal turbinates)		NTP 1987 1,4-dichlorobenzene	
			Cardio	1500				
			Gastro	900		1200 (epithelial necrosis of small intestine mucosa)		
			Hemato	300 F	300[b] M (slight decreases in RBC, HCT, and hemoglobin concentration)			
					600 F (decrease in mean corpuscular volume)			
			Musc/skel	1500				
			Hepatic	300[b] M 900 F	600 M (significant increase in serum cholesterol)	1200 (degeneration and necrosis of hepatocytes)		
			Renal	1500 F		300 M (necrosis of renal cortical tubular epithelium)		
			Endocr	1500				
			Dermal	1500				
			Ocular	900[b] M	1200[b] M (ocular discharge)			
				1200 F	1500 F (ocular discharge)			
			Bd Wt	900 F	300[b] M (11% decrease in final body weight)			
					1200 F (11% decrease in final body weight)			

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
40	Mouse (B6C3F1)	13 wk 5 d/wk (GO)	Hepatic	300	600 (increased liver weight; hypertrophic centrilobular hepatocytes)		Eldridge et al. 1992 1,4-dichlorobenzene	
			Bd Wt	600				
41	Mouse (B6C3F1)	4 or 13 wk 5 d/wk 1 x/d (GO)	Hepatic		300 M (increased relative liver weight; induction of microsomal 7-pentoxyresorufin O-dependentylase activity)		Lake et al. 1997 1,4-dichlorobenzene	
			Renal	600 M				
			Bd Wt	600 M				

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
42	Mouse (B6C3F1)	13 wk 5 d/wk (GO)	Resp	1800			NTP 1987 1,4-dichlorobenzene	
			Cardio	1800				
			Gastro	1800				
			Hemato	1800 F	600 M	(34% reduced WBC count)		
			Musc/skel	1800				
			Hepatic		600	(hepatocellular degeneration in 7/10 males and 9/10 females)		
			Renal	1800				
			Endocr	1800				
			Dermal	1800				
			Ocular	1800				
			Bd Wt		600	(final body weight reduced 13.9% in males and 10.3% in females)		

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
43	Mouse (B6C3F1)	13 wk 5 d/wk (GO)	Resp	900			NTP 1987 1,4-dichlorobenzene	
			Cardio	900				
			Gastro	900				
			Hemato	900				
			Musc/skel	900				
			Hepatic	338	675 (moderate hepatocytomegaly in males and females)			
			Renal	900				
			Endocr	900				
			Dermal	900				
			Ocular	900				
			Bd Wt	900				
44	Dog	6 mo 5 d/wk 1 x/d (C)	Hemato	75			Naylor and Stout 1996 1,4-dichlorobenzene	
			Hepatic	10[c]	50 (increased serum alkaline phosphatase)			
			Bd Wt	75				

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
45	Rabbit (NS)	219 d 92 doses (GO)	Hepatic		1000 (cloudy swelling, very few areas of focal necrosis)		Hollingsworth et al. 1956 1,4-dichlorobenzene	
			Bd Wt		1000 (weight loss, not quantified)			
Immuno/ Lymphoret								
46	Rat (Fischer- 344)	13 wk 5 d/wk (GO)		900		1200 (lymphoid depletion of thymus and spleen)	NTP 1987 1,4-dichlorobenzene	
47	Mouse (B6C3F1)	13 wk 5 d/wk (GO)		1000		1500 (lymphoid necrosis in thymus; lymphoid depletion in the spleen; hematopoietic hypoplasia in spleen and bone marrow)	NTP 1987 1,4-dichlorobenzene	
Neurological								
48	Rat (Fischer- 344)	13 wk 5 d/wk (GO)		900[b] M 1200 F		1200[b] M (tremors, poor motor response) 1500 F (tremors, poor motor response)	NTP 1987 1,4-dichlorobenzene	
49	Rabbit (NS)	219 d 92 doses (GO)				1000 (marked tremors)	Hollingsworth et al. 1956 1,4-dichlorobenzene	

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
50	Rat (Sprague-Dawley)	77-156 d 7 d/wk premating-lactation, two generations (GO)		270			Bornatowicz et al. 1994 1,4-dichlorobenzene	
51	Rat (Wistar)	42 d Gd 1- pnd 21 (F)		2 F			Makita et al. 2004, 2005 1,4-dichlorobenzene	
52	Rat (Fischer- 344)	13 wk 5 d/wk (GO)		1500			NTP 1987 1,4-dichlorobenzene	
53	Mouse (B6C3F1)	13 wk 5 d/wk (GO)		1800 M 1000 F[b]	1500 F (increase in relative ovary weight)		NTP 1987 1,4-dichlorobenzene	
Developmental								
54	Rat (Sprague-Dawley)	77-156 d 7 d/wk premating-lactation, two generations (GO)		30 F		90 F (increased postnatal/preweaning mortality in F1 and F2 pups)	Bornatowicz et al. 1994 1,4-dichlorobenzene	
55	Rat (Wistar)	42 d Gd 1- pnd 21 (F)		2			Makita et al. 2004, 2005 1,4-dichlorobenzene	

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
CHRONIC EXPOSURE								
Death								
56	Rat (Fischer- 344)	2 yr 5 d/wk (GO)				300 M (26/50 deaths)	NTP 1987 1,4-dichlorobenzene	
57	Dog	3 wk 5 d/wk 1 x/d (C)				150 (3/6 deaths)	Naylor and Stout 1996 1,4-dichlorobenzene	
58	Rabbit (NS)	367 d 5 d/wk (GO)				500 (some deaths; not quantified)	Hollingsworth et al. 1956 1,4-dichlorobenzene	

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
59	Rat (Fischer- 344)	2 yr 5 d/wk (GO)	Resp	300[b] M 600 F			NTP 1987 1,4-dichlorobenzene	
			Cardio	300[b] M 600 F				
			Gastro	300[b] M 600 F				
			Hemato	300[b] M 600 F				
			Musc/skel	300[b] M 600 F				
			Hepatic	300[b] M 600 F				
			Renal		150 M	(moderate nephropathy)		
			Endocr	600 F	150 M	(parathyroid hyperplasia)		
			Dermal	300[b] M 600 F				
			Ocular	300[b] M 600 F				
			Bd Wt	150[b] M 300 F	300[b] M	(12.5% decrease in body weight gain)		
					600 F	(12.4% decrease in body weight gain)		

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
60	Mouse (B6C3F1)	2 yr 5 d/wk (GO)	Resp	600			NTP 1987 1,4-dichlorobenzene	
			Cardio	600				
			Gastro	600				
			Hemato	600				
			Musc/skel	600				
			Hepatic		300 (hepatocellular degeneration, hepatocyte swelling and vacuolation)			
			Renal			300 (nephropathy, degeneration of cortical tubular epithelium)		
			Endocr	600 F	300 M (follicular cell hyperplasia in thyroid; adrenal medullary hyperplasia; focal hyperplasia of adrenal gland capsule)			
			Dermal	600				
			Ocular	600				
			Bd Wt	600				

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
61	Dog	1 yr 5 d/wk 1 x/d (C)	Resp	75			Naylor and Stout 1996 1,4-dichlorobenzene	
			Cardio	75				
			Gastro	75				
			Hemato	50	75 (significantly reduced RBC in females and HCT)			
			Musc/skel	75				
			Hepatic	10[d]	50 (increases in serum alkaline phosphatase and liver weight, hepatocellular hypertrophy)			
			Renal	10	50 (suggestive collecting duct epithelial vacuolation)			
			Endocr	75				
			Dermal	75				
			Ocular	75				
			Bd Wt	75				

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
62	Rabbit (NS)	367 d 5 d/wk (GO)	Hepatic		500 (cloudy swelling, some areas of focal necrosis)		Hollingsworth et al. 1956 1,4-dichlorobenzene	
			Bd Wt		500 (weight loss, not quantified)			
Immuno/ Lymphoret								
63	Rat (Fischer- 344)	2 yr 5 d/wk (GO)		600			NTP 1987 1,4-dichlorobenzene	
64	Mouse (B6C3F1)	2 yr 5 d/wk (GO)			300 (lymphoid hyperplasia of lymph nodes)		NTP 1987 1,4-dichlorobenzene	
65	Dog	1 yr 5 d/wk 1 x/d (C)		75			Naylor and Stout 1996 1,4-dichlorobenzene	
Neurological								
66	Rat (Fischer- 344)	2 yr 5 d/wk (GO)		600			NTP 1987 1,4-dichlorobenzene	
67	Mouse (B6C3F1)	2 yr 5 d/wk (GO)		600			NTP 1987 1,4-dichlorobenzene	
68	Rabbit (NS)	367 d 5 d/wk (GO)				500 (marked tremors)	Hollingsworth et al. 1956 1,4-dichlorobenzene	

Table 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
69	Rat (Fischer- 344)	2 yr 5 d/wk (GO)		600			NTP 1987 1,4-dichlorobenzene	
70	Mouse (B6C3F1)	2 yr 5 d/wk (GO)		600			NTP 1987 1,4-dichlorobenzene	
Cancer								
71	Rat (Fischer- 344)	2 yr 5 d/wk (GO)				300 M (CEL: renal tubular cell adenoma and adenocarcinoma)	NTP 1987 1,4-dichlorobenzene	
72	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				600 (CEL: hepatocellular adenoma and carcinoma)	NTP 1987 1,4-dichlorobenzene	

a The number corresponds to entries in Figure 3-5

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-5. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c Study result used to derive an intermediate-duration oral minimal risk level (MRL) of 0.07 mg/kg/day for 1,4-DCB, as described in detail in Appendix A. Benchmark dose analysis was performed on serum alkaline phosphatase levels to select a point of departure, which was duration adjusted, then divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

d Study result used to derive a chronic-duration oral minimal risk level (MRL) of 0.07 mg/kg/day for 1,4-DCB, as described in detail in Appendix A. Benchmark dose analysis was performed on incidences of serum alkaline phosphatase levels to select a point of departure, which was duration adjusted, then divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

ALT = alamine aminotransferase; Bd Wt = body weight; BrdU = Bromodeoxyuridine; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; EPN = O-ethyl O-p-nitrophenyl phenylphosphonothioate; (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; Gn pig = guinea pig; (GO) = gavage in oil; HCT = hematocrit; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LD100 = lethal dose, 100% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; pnd = post-natal day; RBC = red blood cell; Resp = respiratory; x = time(s); WBC = white blood cell; wk = week(s); yr = year(s)

Figure 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral

Acute (≤14 days)

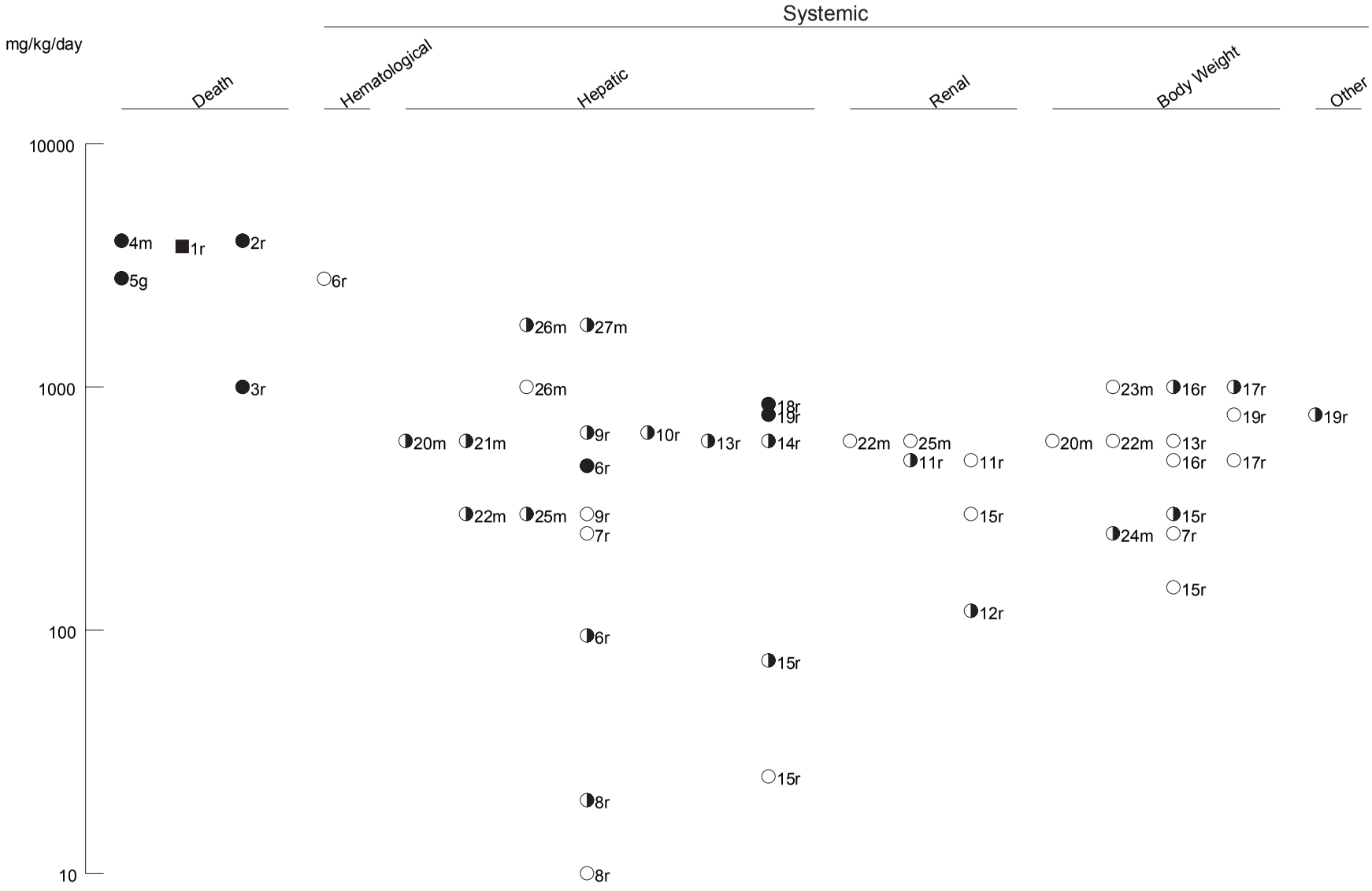


Figure 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral *(Continued)*

Acute (≤14 days)

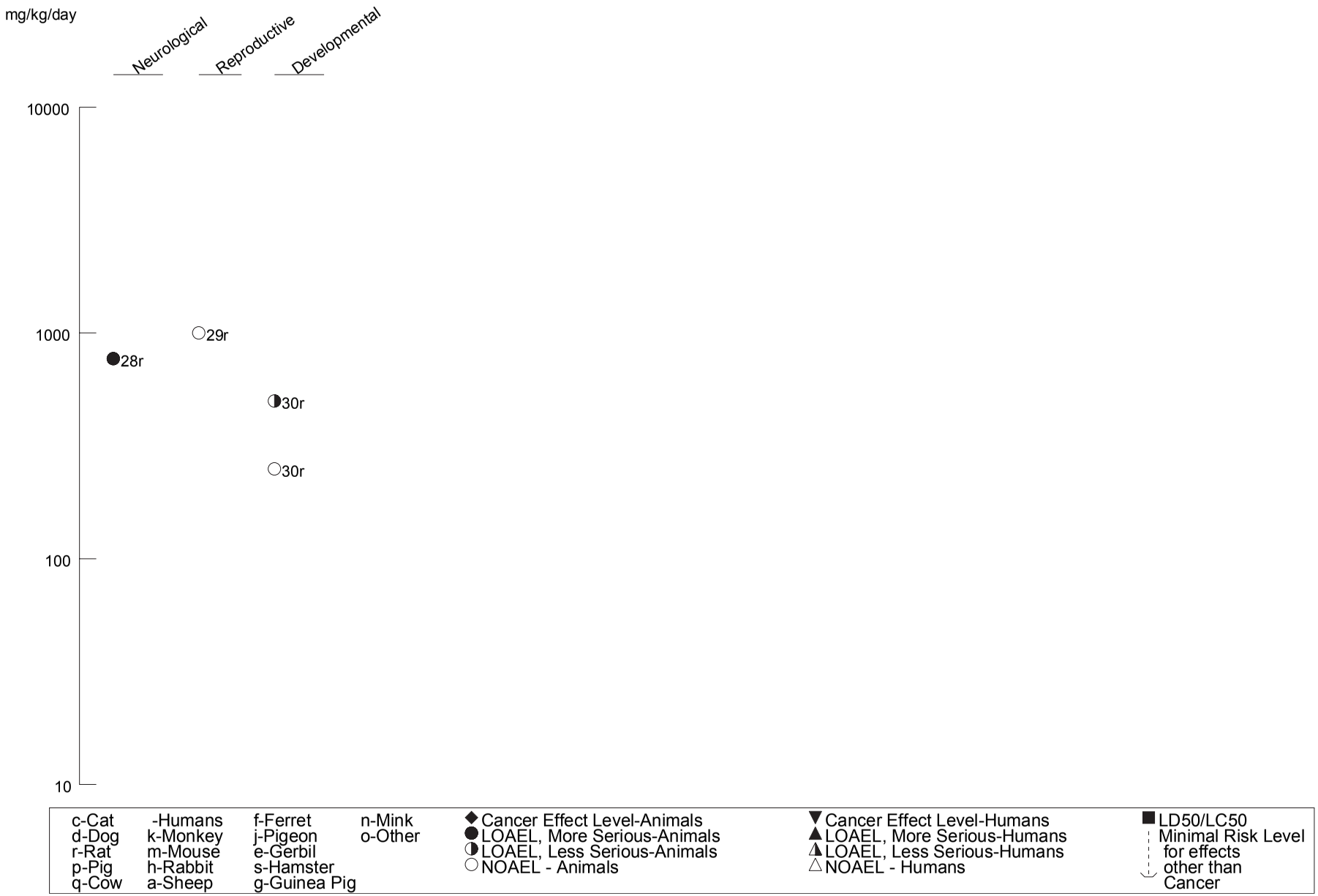


Figure 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

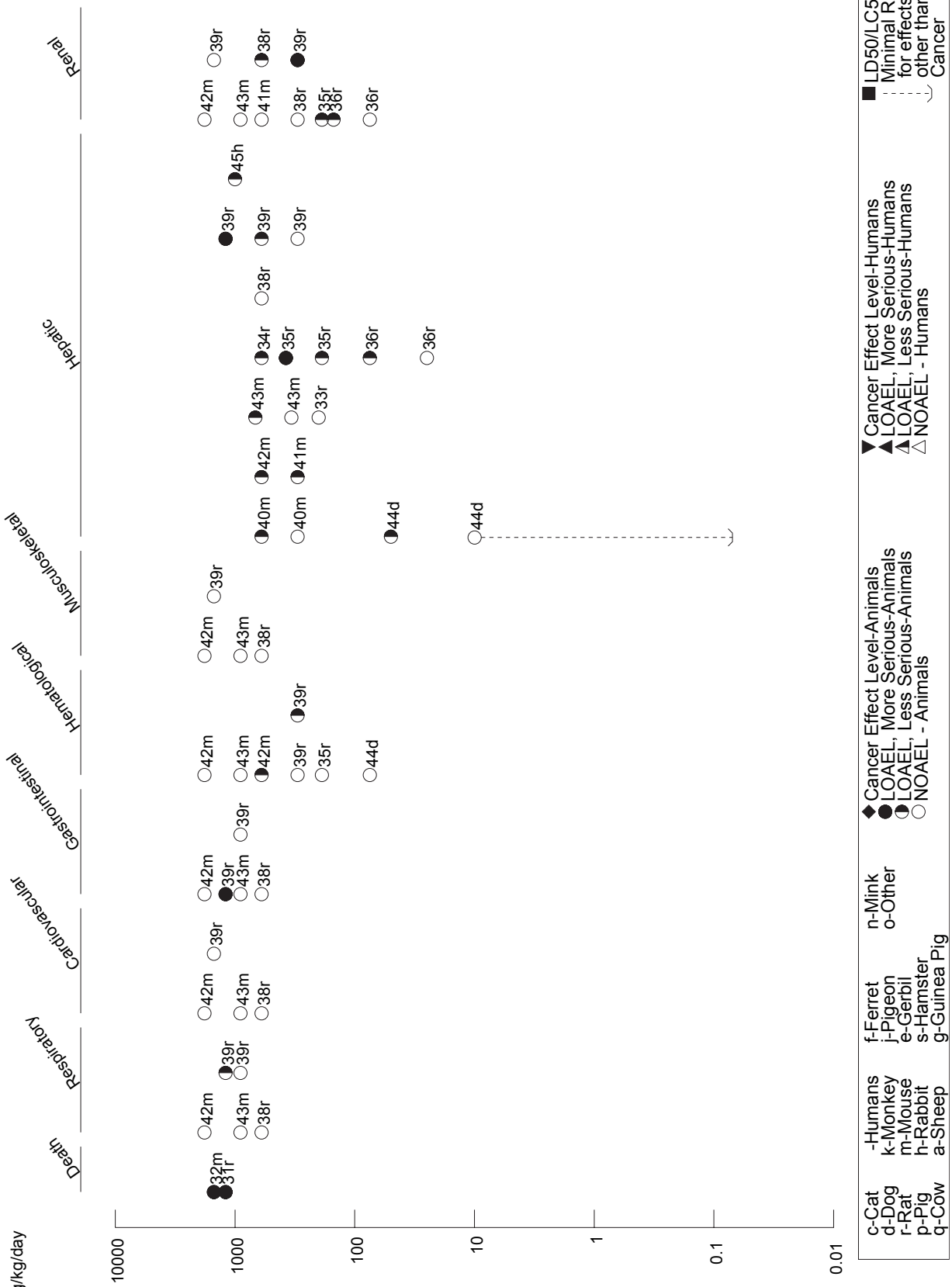


Figure 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral *(Continued)*

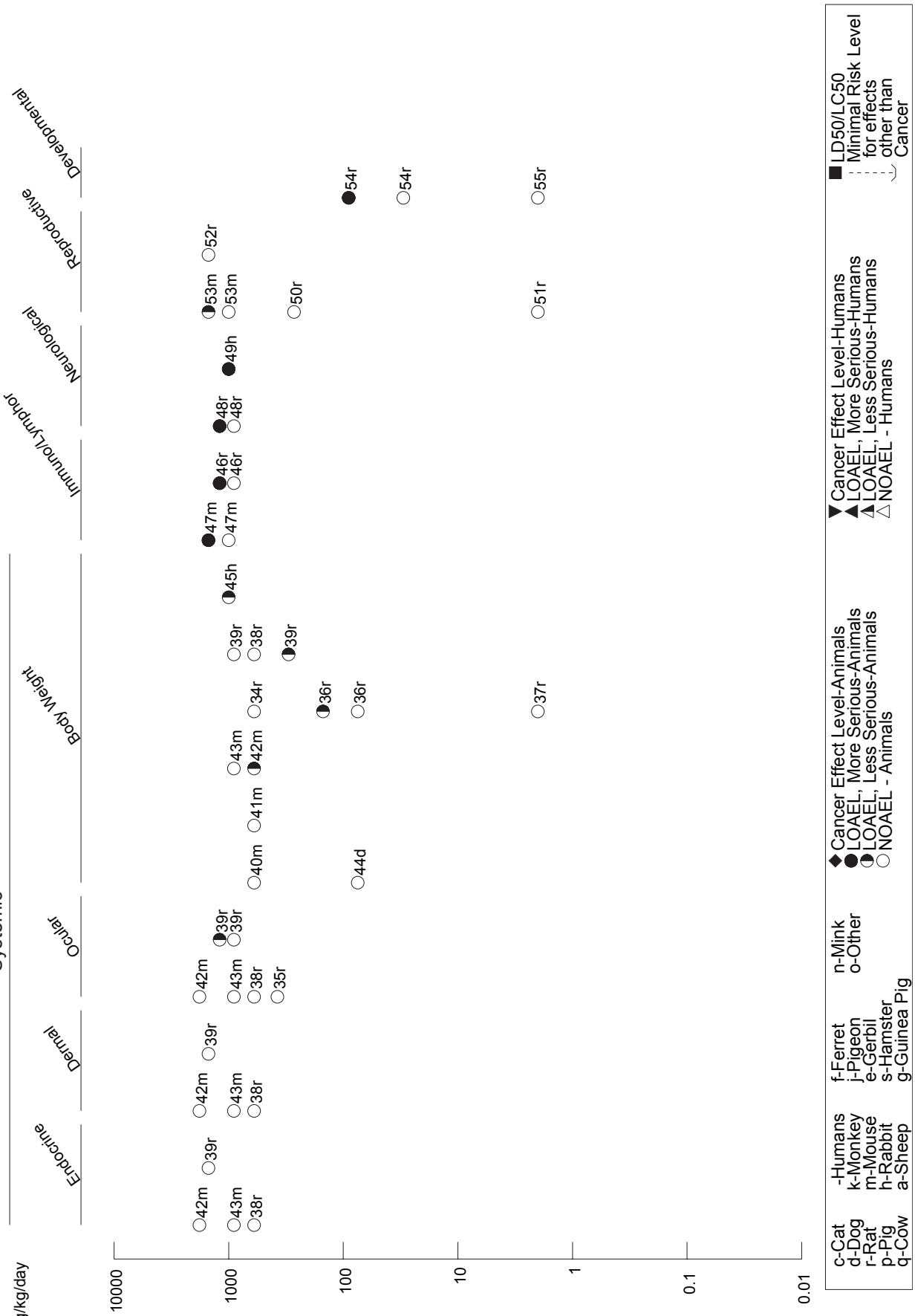


Figure 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral *(Continued)*

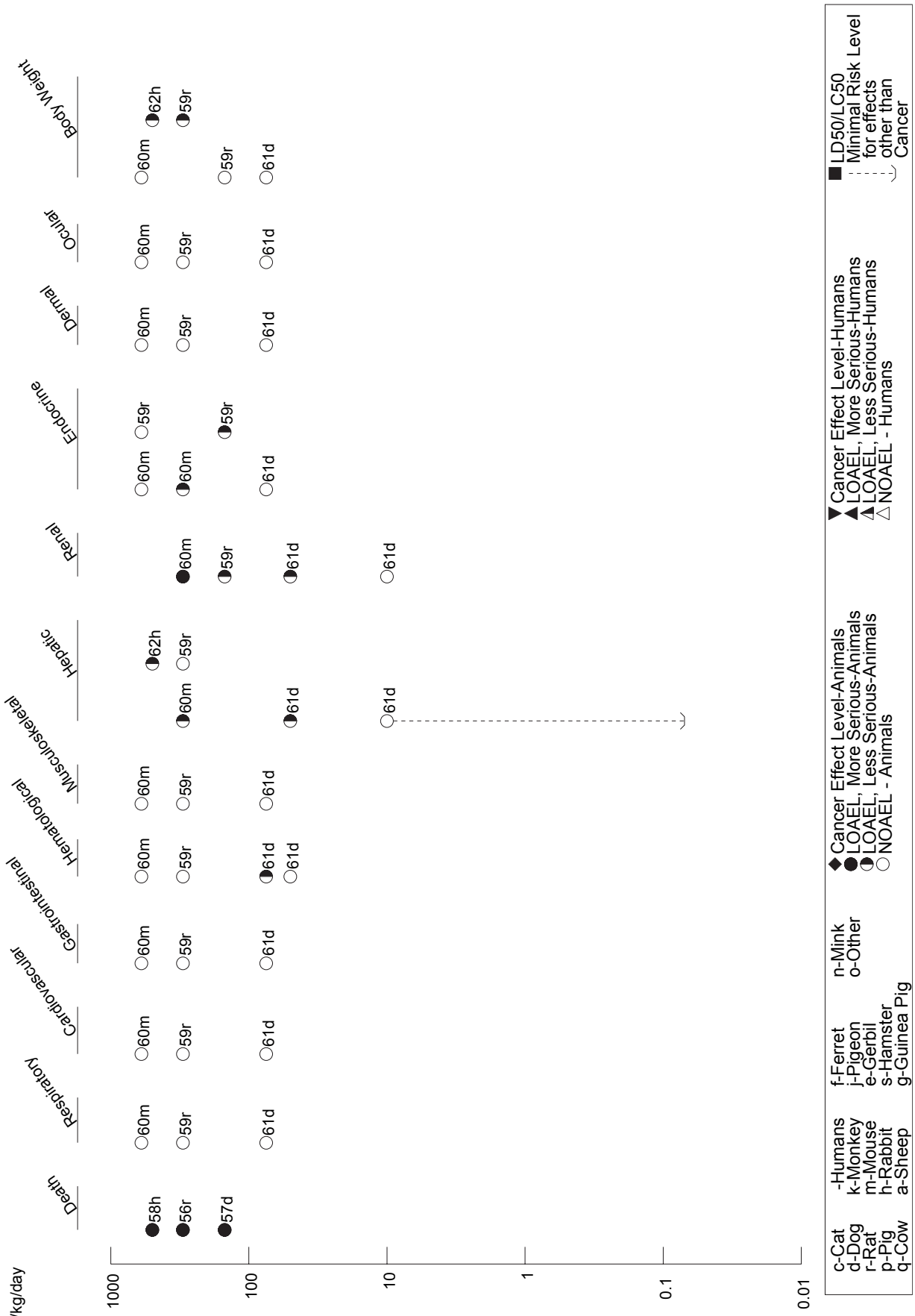
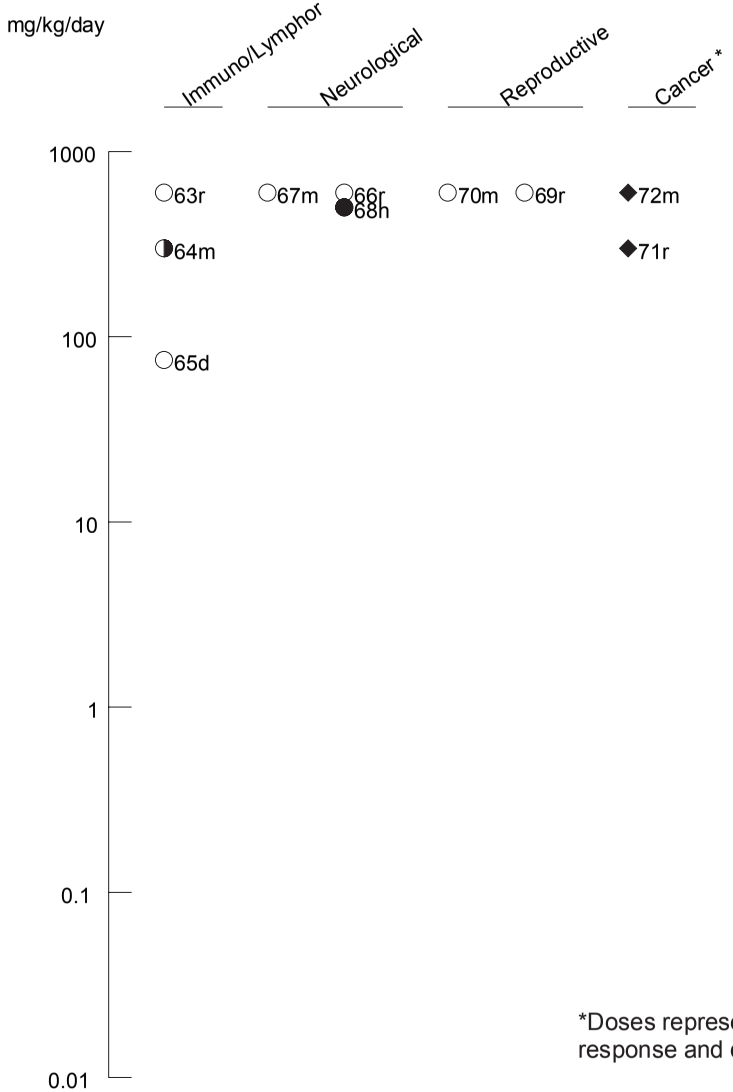


Figure 3-5 Levels of Significant Exposure to 1,4-dichlorobenzene - Oral *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat d-Dog r-Rat p-Pig q-Cow
-Humans k-Monkey m-Mouse h-Rabbit a-Sheep
f-Ferret j-Pigeon e-Gerbil s-Hamster g-Guinea Pig
n-Mink o-Other

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Acute oral LD_{50} values of 1,200 and 1,000 mg/kg were determined in male and female Sprague-Dawley rats, respectively, administered a single dose of 1,3-DCB by gavage and observed for the following 14 days (Monsanto 1980).

No mortality or overt signs of toxicity occurred in male or female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by gavage in doses as high as 735 mg/kg/day for 10 consecutive days, or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995).

1,4-Dichlorobenzene. No studies were located regarding death in humans after oral exposure to 1,4-DCB.

Animal mortality data for 1,4-DCB are available from acute-, intermediate-, and chronic-duration studies. In acute-duration animal studies, a single dose by gavage in olive oil of 1,000 mg/kg to rats and 1,600 mg/kg to guinea pigs resulted in no deaths, while a single dose of 4,000 mg/kg to rats and 2,800 mg/kg to guinea pigs resulted in 100% mortality (Hollingsworth et al. 1956). Similar results were seen in groups of adult male albino rats administered various doses of 1,4-DCB in corn oil once daily for 14 days; administration of 1,4-DCB at doses up to 600 mg/kg did not result in any deaths (Carlson and Tardiff 1976). Oral LD_{50} (lethal dose, 50% kill) values for adult Sherman rats administered 1,4-DCB in peanut oil were calculated to be 3,863 and 3,790 mg/kg for males and females, respectively (Gaines and Linder 1986). In contrast, groups of male F344 rats (n=1/group) were administered 13–27,900 mg/kg body weight in corn oil via gavage. Twenty-four hours after dosing, the animals were weighed and exsanguinated. No mortality among the 1,4-DCB-treated rats was observed (Allis et al. 1992).

In one series of studies (NTP 1987), the lethality data for 1,4-DCB, when administered for 14 days by gavage in corn oil to F344 rats and $B6C3F_1$ mice, were rather inconsistent. In one of these studies, no 1,4-DCB-related deaths occurred in rats of either sex that received doses up to 1,000 mg/kg/day; however, in the second rat study, four of five females (80%) at 1,000 mg/kg/day died, and all rats dosed at >2,000 mg/kg/day died. In one 14-day study in mice, no 1,4-DCB-related deaths occurred in either sex at levels up to 1,000 mg/kg/day; however, in a second 14-day mouse study, 70% of mice at 1,000 mg/kg/day died, and all mice that received 4,000 mg/kg/day died within 4 days. At 1,200 mg/kg/day, 5 of 10 male and 1 of 10 female rats died. No deaths occurred at 600 mg/kg/day.

In 13-week gavage studies, 17 of 20 rats (8 of 10 males and 9 of 10 females) dosed with 1,4-DCB in corn oil 5 days/week at 1,500 mg/kg/day died. When dosed in like manner with 1,200 mg/kg/day, 5 of

10 male and 1 of 10 female rats died. No deaths occurred at doses of ≤600 mg/kg/day (NTP 1987). Mortality rates in mice were somewhat lower; 8 of 20 (3 of 10 males and 5 of 10 females) animals dosed with 1,500 mg/kg/day 1,4-DCB in corn oil 5 days/week died. No deaths occurred in males or females at doses up to 900 and 1,000 mg/kg/day, respectively (NTP 1987).

High mortality was reported in male rats that received 1,4-DCB 5 days/week by gavage in corn oil in a 2-year study (NTP 1987). At 300 mg/kg/day, 26 of 50 males (52%) died; however, survival of female rats at 600 mg/kg/day was comparable to controls. There was no excess mortality in mice of either sex that received 1,4-DCB 5 days/week by gavage in corn oil for 2 years at levels up to 600 mg/kg/day (NTP 1987). The high rate of mortality in male rats was probably related, in part, to the severe nephrotoxic effects and renal tumors that were reported in these animals and are described in more detail in Sections 3.2.2.2 and 3.2.2.7.

Groups of five male and five female Beagle dogs were administered 1,4-DCB by capsule in dose levels of 0, 10, 50, or 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996). The 75 mg/kg/day dose is a time-weighted average level reflecting decreases from an initial high level of 150 mg/kg/day in response to severe toxicity. The main early effect was mortality during the first 25 days of the study; exposure to 150 mg/kg/day caused one male dog to be sacrificed *in extremis* on day 12, one male death on day 25, and one female death on day 24. With the exception of one control male that died on day 83, all remaining dogs survived exposure to 75 mg/kg/day.

3.2.2.2 Systemic Effects

Respiratory Effects.

1,2-Dichlorobenzene. No studies were located regarding respiratory effects in humans after oral exposure to 1,2-DCB.

No gross or histological changes were observed in the respiratory tract (nasal cavity, trachea, lungs, and/or bronchi) of Sprague-Dawley or F344 rats that were administered 1,2-DCB in corn oil by gavage in doses of 300 mg/kg/day for 10 consecutive days (Robinson et al. 1991), 400 mg/kg/day for 90 consecutive days (Robinson et al. 1991), ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985), or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985). There were no gross or histological effects in

the respiratory system of B6C3F$_1$ mice that were similarly treated with ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985), or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985).

1,3-Dichlorobenzene. No studies were located regarding respiratory effects in humans after oral exposure to 1,3-DCB.

No gross or histological changes were observed in the respiratory tract (nasal cavity and turbinates, lungs, and lower half of trachea) in male or female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by gavage in doses of 735 mg/kg/day for 10 consecutive days or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995).

1,4-Dichlorobenzene. No studies were located regarding respiratory effects in humans after oral exposure to 1,4-DCB.

In a series of dose range-finding studies, groups of F344 rats were administered 1,4-DCB at concentrations ranging from 37.5 to 1,500 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks (NTP 1987). At sacrifice, animals were examined grossly and major tissues were examined histologically. No compound-related effects were observed in the lungs at any dose up to 900 mg/kg/day, while rats treated with 1,200 mg/kg/day or higher exhibited epithelial necrosis of the nasal turbinates (NTP 1987). In parallel studies, B6C3F$_1$ mice were administered 1,4-DCB at concentrations ranging from 84.4 to 1,800 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks. No compound-related effects were observed in the lungs at any dose level (NTP 1987).

In 2-year exposure studies in F344 rats, no respiratory effects were reported in males or females that received 1,4-DCB by gavage in corn oil at levels up to 300 or 600 mg/kg/day, respectively (NTP 1987). In similarly dosed B6C3F$_1$ mice, no respiratory effects were reported in either sex at doses up to 600 mg/kg/day (NTP 1987).

Cardiovascular Effects.

1,2-Dichlorobenzene. No studies were located regarding cardiovascular effects in humans after oral exposure to 1,2-DCB.

Mutifocal mineralization of the myocardial fibers of the heart (as well as skeletal muscle) was found in $B6C3F_1$ mice that were administered 500 mg/kg/day of 1,2-DCB in corn oil by gavage 5 days/week for 13 weeks (NTP 1985); this effect does not appear to have occurred in controls or lower dose groups (≤250 mg/kg/day). No gross or histological changes were observed in the heart of $B6C3F_1$ mice that were similarly treated with ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985), or in Sprague-Dawley or F344 rats that were similarly treated with 300 mg/kg/day for 10 consecutive days (Robinson et al. 1991), 400 mg/kg/day for 90 consecutive days (Robinson et al. 1991), ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985), or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985).

1,3-Dichlorobenzene. No studies were located regarding cardiovascular effects in humans after oral exposure to 1,3-DCB.

No gross or histological changes in the aorta were observed in male or female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by gavage in doses of 735 mg/kg/day for 10 consecutive days or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995).

1,4-Dichlorobenzene. No studies were located regarding cardiovascular effects in humans after oral exposure to 1,4-DCB.

In a series of dose range-finding studies, groups of F344 rats were administered 1,4-DCB at concentrations ranging from 37.5 to 1,500 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks (NTP 1987). At sacrifice, animals were examined grossly and major tissues were examined histologically. No compound-related cardiovascular effects were observed at any dose level. In parallel studies, $B6C3F_1$ mice were administered 1,4-DCB at concentrations ranging from 84.4 to 1,800 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks. As with the rats, no compound-related cardiovascular effects were observed in mice at any of the doses used (NTP 1987).

In 2-year exposure studies in F344 rats, no cardiovascular effects were reported in males or females that received 1,4-DCB by gavage in corn oil at levels up to 300 or 600 mg/kg/day, respectively (NTP 1987). In similarly dosed $B6C3F_1$ mice, no cardiovascular effects were reported in either sex at doses up to 600 mg/kg/day (NTP 1987).

No gross or histological changes were found in the aorta or heart of Beagle dogs (5/sex/level) that were administered 1,4-DCB by capsule in doses as high as 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996).

Gastrointestinal Effects.

1,2-Dichlorobenzene. No studies were located regarding gastrointestinal effects in humans after oral exposure to 1,2-DCB.

No gross or histological changes were observed in the gastrointestinal tract (esophagus, stomach, small intestine, colon, and/or other tissues) of Sprague-Dawley or F344 rats that were administered 1,2-DCB in corn oil by gavage in doses of 300 mg/kg/day for 10 consecutive days (Robinson et al. 1991), ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985), or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985). Additionally, there were no gross or histological effects in the gastrointestinal tract of $B6C3F_1$ mice that were similarly treated with ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985), or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985).

1,3-Dichlorobenzene. No studies were located regarding gastrointestinal effects in humans after oral exposure to 1,3-DCB.

No gross or histological changes were observed in the gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, colon, cecum, rectum, tongue) in male or female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by gavage in doses of 735 mg/kg/day for 10 consecutive days, or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995).

1,4-Dichlorobenzene. No studies were located regarding gastrointestinal effects in humans after oral exposure to 1,4-DCB.

In a series of dose range-finding studies, groups of F344 rats were administered 1,4-DCB at concentrations ranging from 37.5 to 1,500 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks (NTP 1987). At sacrifice, animals were examined grossly and major tissues were examined histologically. Gastrointestinal effects were observed at doses of 1,200 mg/kg/day or more and consisted of epithelial necrosis and villar bridging of the mucosa of the small intestines. No gastrointestinal effects were noted in rats treated with 1,4-DCB at doses of 900 mg/kg/day or less (NTP 1987). In parallel

studies with B6C3F_1 mice, no compound-related gastrointestinal effects were observed after administration of 1,4-DCB at concentrations ranging from 84.4 to 1,800 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks (NTP 1987).

In 2-year exposure studies in Fischer 344 rats, no gastrointestinal effects were reported in males or females that received 1,4-DCB by gavage in corn oil at levels up to 300 or 600 mg/kg/day, respectively (NTP 1987). In similarly dosed B6C3F_1 mice, no gastrointestinal effects were reported in either sex at doses up to 600 mg/kg/day (NTP 1987).

No gross or histological changes were found in the gastrointestinal tract of Beagle dogs (5/sex/level) that were administered 1,4-DCB by capsule in doses as high as 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996). Nine regions of the gastrointestinal tract were examined.

Hematological Effects.

1,2-Dichlorobenzene. No studies were located regarding hematological effects in humans after oral exposure to 1,2-DCB.

No hematological changes were observed in Sprague-Dawley or F344 rats that were administered 1,2-DCB in corn oil by gavage in doses of ≤300 mg/kg/day for 10 consecutive days (Robinson et al. 1991), ≤400 mg/kg/day for 90 consecutive days (Robinson et al. 1991), or ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985). Additionally, there were no hematological effects in B6C3F_1 mice that were similarly treated with ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985).

1,3-Dichlorobenzene. No studies were located regarding hematological effects in humans after oral exposure to 1,3-DCB.

No hematological changes (numbers of erythrocytes and leukocytes, hemoglobin level, hematocrit, or mean corpuscular volume) were observed in male or female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by gavage in doses of 735 mg/kg/day for 10 consecutive days, or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995).

1,4-Dichlorobenzene. A 21-year-old pregnant woman who had eaten 1–2 blocks of 1,4-DCB toilet air freshener per week throughout pregnancy developed severe microcytic, hypochromic anemia with

excessive polychromasia and marginal nuclear hypersegmentation of the neutrophils. Heinz bodies were seen in a small number of the red cells. After she discontinued this practice (at about 38 weeks of gestation), her hemoglobin levels began to rise steadily. She gave birth to a normal infant with no hematological problems, and her own red blood cells were again normal at the final check 6 weeks after delivery (Campbell and Davidson 1970). Acute hemolytic anemia and were reported to have occurred in a 3-year-old boy who had played with 1,4-DCB crystals (Hallowell 1959). It is not clear whether this child had actually ingested any of the 1,4-DCB crystals.

Hematological effects reported in animal studies mainly concern effects on red cells in rats and on white cells in mice. Groups of male F344 rats (n=1/group) were administered 13–2,790 mg/kg body weight of 1,4-DCB once via corn oil gavage. Twenty-four hours after dosing, the animals were weighed and exsanguinated. No hematological alterations were noted in any of the treated rats (Allis et al. 1992).

No adverse effects on hemoglobin levels or hematocrit were seen in adult male albino rats dosed with 1,4-DCB by gavage in corn oil at levels up to 40 mg/kg/day for 90 days (Carlson and Tardiff 1976).

In F344 rats administered 1,4-DCB by gavage in corn oil, 7 days/week for 13 weeks at doses of 75–600 mg/kg/day, no compound-related hematological effects were noted (Bomhard et al. 1988). In a series of experiments performed by Hollingsworth et al. (1956), male rats were administered 1,4-DCB by gavage in olive oil at doses of 10–500 mg/kg/day, 5 days/week for 4 weeks; female rats received 1,4-DCB in like manner at doses of 18.8–376 mg/kg/day, 5 days/week for 192 days; and male and female rabbits received 500 mg/kg/day 1,4-DCB, 5 days/week for 367 days. Administration of 1,4-DCB produced no hematological effects at any dose.

In another 13-week study in F344 rats, male rats that received 1,4-DCB at 300 mg/kg/day and above had decreased hematocrit levels, red blood cell counts, and hemoglobin concentrations (NTP 1987). None of these hematologic effects were consistently seen in female rats at the same dosage level; however, a decrease in mean corpuscular volume was noted in females at doses of 600 mg/kg/day or more. In a parallel study in male and female B6C3F_1 mice dosed with 84.4–900 mg/kg/day 1,4-DCB for 13 weeks, no hematological effects were noted in male or female mice at doses up to 900 mg/kg/day (NTP 1987); however, in another study, B6C3F_1 mice dosed with 600–1,800 mg/kg/day 1,4-DCB for 13 weeks showed hematologic effects including 34–50% reductions in the white cell counts in all male dose groups; these decreases were accompanied by 26–33% decreases in lymphocytes and 69–82% decreases in neutrophils. No hematological effects were noted in female B6C3F_1 mice at doses up to 1,800 mg/kg/day (NTP 1987).

No hematologic effects were reported in 2-year studies in which male F344 rats received 1,4-DCB at levels up to 300 mg/kg/day/day and female rats received levels up to 600 mg/kg/day (NTP 1987). Similar results were reported in $B6C3F_1$ mice of both sexes exposed to 600 mg/kg/day 1,4-DCB for 2 years (NTP 1987).

Hematology was evaluated in groups of five male and five female Beagle dogs that were administered 1,4-DCB by capsule in doses of 0, 10, 50, or 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996). Ten routine indices and one blood clotting measurement (activated partial thromboplastin time) were evaluated at 6 and 12 months. A mild anemia, as indicated by significantly reduced red blood cell count in females and hematocrit in males, was observed after 6 months at 75 mg/kg/day, but resolved by the end of the study. Histological findings in the bone marrow (erythroid hyperplasia in females) and spleen (excessive hematopoiesis and megakaryocyte proliferation in both sexes) at 75 mg/kg/day indicated a compensatory response to the earlier anemia.

Musculoskeletal Effects.

1,2-Dichlorobenzene. No studies were located regarding musculoskeletal effects in humans after oral exposure to 1,2-DCB.

Mutifocal mineralization of the myocardial fibers of the heart and skeletal muscle was found in $B6C3F_1$ mice (3/10 males, 8/10 females) that were administered 500 mg/kg/day of 1,2-DCB in corn oil by gavage 5 days/week for 13 weeks (NTP 1985); this effect does not appear to have occurred in controls or lower dose mice (≤250 mg/kg/day). No gross or histological changes were observed in muscle of $B6C3F_1$ mice that were similarly treated with ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985), or in Sprague-Dawley or F344 rats that were similarly treated with 300 mg/kg/day for 10 consecutive days (Robinson et al. 1991), ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985), or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985).

No gross or histological changes in bone were observed in any of the rat or mouse 10-day, 13-week, or 103-week studies summarized above (NTP 1985; Robinson et al. 1991).

1,3-Dichlorobenzene. No studies were located regarding musculoskeletal effects in humans after oral exposure to 1,3-DCB.

No gross or histological changes were observed in thigh muscle or sternebrae in male or female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by gavage in doses of 735 mg/kg/day for 10 consecutive days or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995).

1,4-Dichlorobenzene. No studies were located regarding musculoskeletal effects in humans after oral exposure to 1,4-DCB.

In a series of dose range-finding studies, groups of F344 rats were administered 1,4-DCB at concentrations ranging from 37.5 to 1,500 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks. At sacrifice, animals were examined grossly and major tissues were examined histologically. No musculoskeletal effects were noted in any of the 1,4-DCB-treated rats. In parallel studies with $B6C3F_1$ mice, no compound-related musculoskeletal effects were observed after administration of 1,4-DCB at concentrations ranging from 84.4 to 1,800 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks (NTP 1987).

In 2-year exposure studies in F344 rats, no musculoskeletal effects were reported in males or females that received 1,4-DCB by gavage in corn oil at levels up to 300 or 600 mg/kg/day, respectively. In similarly dosed $B6C3F_1$ mice, no musculoskeletal effects were reported in either sex at doses up to 600 mg/kg/day (NTP 1987).

No gross or histological changes were found in skeletal muscle or bone of Beagle dogs (5/sex/level) that were administered 1,4-DCB by capsule in doses as high as 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996).

Hepatic Effects.

1,2-Dichlorobenzene. No studies were located regarding hepatic effects in humans after oral exposure to 1,2-DCB.

The liver is a main target of toxicity in animals following oral exposure to 1,2-DCB. Necrosis and other degenerative hepatic changes were observed in acute-duration studies in which 1,2-DCB was administered in oil by gavage. A single 1,500 mg/kg dose (a lethal level) caused central necrosis of the liver in rats (number and gender not reported) (DuPont 1982). Severe liver damage, characterized by

intense necrosis and fatty changes, occurred in three male rats administered 455 mg/kg/day for 15 consecutive days (Rimington and Ziegler 1963). Other hepatic effects in this study included porphyria, manifested as increased mean peak urinary levels of coproporphyrin, uroporphyrin, porphobilinogen (PBG), and γ-aminolevulinic acid (ALA) that were approximately 10-fold higher than levels in controls. Liver changes in other acute-duration studies included necrosis and increased serum ALT in rats given 300 mg/kg/day for 10 consecutive days (Robinson et al. 1991). The necrosis was slight in severity and significantly ($p=0.04$) increased in males at 300 mg/kg/day [4/10 compared to 0/10 in controls; incidences in lower dose groups (37.5, 75, and 150 mg/kg/day) were not specifically reported and are assumed to be 0/10]. Incidences of other hepatic lesions were not significantly increased but included inflammation (characterized by lymphocyte and macrophage infiltrates) and degeneration of hepatocytes (characterized varying degrees of fibrillar or vacuolated cytoplasm and swelling with intact cell membranes). Liver weight was increased in females at ≥150 mg/kg/day and males at 300 mg/kg/day; the increased liver weight in female rats in this study (Robinson et al. 1991) was selected as the critical effect for deriving an acute-duration oral MRL of 0.7 mg/kg/day for 1,2-DCB. No liver histopathology was observed in male or female rats that were given doses as high as 500 or 1,000 mg/kg/day for 14 consecutive days (NTP 1985). The inconsistency between these findings and those of Robinson et al. (1991) might be due to a small number of animals (5 rats/sex/dose level) in the NTP (1985) study and mild response (low incidence and severity of lesions) in the Robinson et al. (1991) study. Hepatic degeneration and necrosis were observed in mice exposed to 250 or 500 mg/kg/day for 14 consecutive days (NTP 1985), but this study is also limited by small numbers of animals (3–4 mice/sex/group).

Liver histopathology was also the predominant finding in intermediate-duration studies of rats and mice exposed to 1,2-DCB (Hollingsworth et al. 1958; NTP 1985; Robinson et al. 1991). The compound was administered in oil vehicle by gavage in all of these studies. Slight to moderate cloudy swelling of the liver was found in female rats (strain not specified) dosed with 376 mg/kg/day, 5 days/week for 138 doses in 192 days, but not at lower dose levels of 18.8 or 188 mg/kg/day (Hollingsworth et al. 1958). The incidence of the lesion was not reported. Liver weight was increased at ≥188 mg/kg/day, but it is unclear whether this is an adaptive change or adverse effect due to the lack of histological or other evidence of tissue damage.

Administration of 400 mg/kg/day for 90 consecutive days caused significantly increased incidences of lesions in Sprague-Dawley rats, including centrilobular degeneration, centrilobular hypertrophy, and single cell necrosis in 10/10, 9/10, and 7/10 males, respectively, and 8/10, 10/10, and 5/10 females, respectively (Robinson et al. 1991). Histology was not evaluated at other dose levels (25 or

100 mg/kg/day), although no lesions occurred in controls of either sex. Absolute and relative liver weights and serum levels of ALT were significantly increased at ≥100 mg/kg/day, but the increases in ALT were not dose-related and other liver-associated enzymes (AST, LDH, AP) were not increased. The 400 mg/kg/day dose is a LOAEL for hepatic effects based on histopathology. A reliable NOAEL cannot be identified because histology was not evaluated at lower doses, the increase in serum ALT was not dose-related or supported by changes in other serum indicators of liver damage, and an increase in liver weight without clear evidence of tissue damage is considered to be an adaptive response.

NTP (1985) conducted subchronic studies in F344/N rats and B6C3F$_1$ mice to determine doses to be used in chronic bioassays. Groups of 10 males and 10 females of each species were administered 1,2-DCB in doses of 0, 30, 60, 125, 250, or 500 mg/kg/day, 5 days/week for 13 weeks. Histology examinations of the liver were limited to the control and three highest dose groups. Degenerative lesions were significantly ($p \leq 0.05$) increased in both species at ≥250 mg/kg/day. Changes in the rats included necrosis of individual hepatocytes at ≥250 mg/kg/day and centrilobular degeneration at 500 mg/kg/day; total incidences of these lesions at 0, 125, 250, and 500 mg/kg/day were 0/10, 1/10, 4/9, and 8/10 in males, respectively, and 0/10, 3/10, 5/10, and 7/8 in females, respectively. Relative liver weight was significantly increased at ≥125 mg/kg/day in both sexes, but there were no increases in serum levels of liver enzymes (ALT, AP, or gamma-glutamyltranspeptidase [GGPT]) at any dose. Serum cholesterol was significantly increased in males at ≥30 mg/kg/day (50.0, 17.6, 26.5, 70.6, and 109% higher than controls in the low to high dose groups, not significant at 42.9 mg/kg/day) and females at ≥125 mg/kg/day (12.2, 12.2, 32.6, 26.5, and 51.0%). Urinary concentrations of uroporphyrin and coproporphyrin were 3–5 times higher than controls in the 500 mg/kg/day males and females, but this increase was not considered indicative of porphyria because total porphyrin concentration in the liver was not altered at any dose level and no pigmentation indicative of porphyria was observed by ultraviolet light at necropsy. The increases in relative liver weight seen in male and female rats at 125 mg/kg/day are believed to represent the beginning of adverse hepatic effects, indicating that 125 mg/kg/day is a minimal LOAEL for this study. The increased liver weight in the female rats in this study (NTP 1985) was selected as the critical effect for deriving an intermediate-duration oral MRL of 0.6 mg/kg/day for 1,2-DCB. In the mice, no compound-related histopathological changes were observed in either sex at 0 and 125 mg/kg/day, or in females at 250 mg/kg/day. Lesions that were significantly increased included necrosis of individual hepatocytes, hepatocellular degeneration and/or pigment deposition in 4/10 males at 250 mg/kg/day, and centrilobular necrosis, necrosis of individual hepatocytes, and/or hepatocellular degeneration in 9/10 males and 9/10 females at 500 mg/kg/day. Relative liver weights were significantly increased at 500 mg/kg/day in both sexes, but there were no exposure-related changes in serum levels of ALT, AP, or GGPT in either

sex at any dose (no other clinical chemistry indices were examined in the mice). The hepatic histopathology findings indicate that the NOAEL and LOAEL for liver effects in mice are 125 and 250 mg/kg/day, respectively.

In the NTP (1985) chronic study, groups of 50 male and 50 female F344/N rats and $B6C3F_1$ mice were administered 1,2-DCB in corn oil by gavage in doses of 0, 60, or 120 mg/kg/day, 5 days/week for 103 weeks. Histopathological examinations were performed in all animals, although liver weights and clinical chemistry indices were not evaluated. There were no exposure-related nonneoplastic liver lesions in either species, indicating that 125 mg/kg/day is the chronic NOAEL for liver effects in both rats and mice.

1,3-Dichlorobenzene. No studies were located regarding hepatic effects in humans after oral exposure to 1,3-DCB.

Liver toxicity was evaluated in groups of 10 male and 10 female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by daily gavage, in doses of 0, 37, 147, 368, or 735 mg/kg/day for 10 consecutive days, or 9, 37, 147, or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995). Study end points included serum chemistry indices (AP, AST, ALT, LDH, cholesterol), liver weight, and gross appearance and histology of the liver. As discussed below, hepatic changes were found at ≥147 mg/kg/day in the 10-day study and ≥9 mg/kg/day in the 90-day study.

Hepatic effects in the 10-day rat study included significantly ($p \leq 0.05$) increased relative liver weight in males at ≥147 mg/kg/day and females at ≥368 mg/kg/day (absolute organ weight not reported), and histopathology at ≥368 mg/kg/day in both sexes. Increased liver weight in this study (McCauley et al. 1995) was selected as the critical effect for deriving an acute-duration oral MRL of 0.4 mg/kg/day for 1,3-DCB. The main hepatic histological change was dose-related centrilobular hepatocellular degeneration, characterized by varying degrees of cytoplasmic vacuolization and swelling with intact membranes. Respective incidences of this lesion at 368 and 735 mg/kg/day were 2/10 and 9/10 in males, and 6/10 and 10/10 females; incidences in the other groups were not reported, but are presumed to be 0/10. Other hepatic alterations included hepatocellular necrosis that was sporadically noted in the 147, 368, and 735 mg/kg/day groups. This change was usually minimal to mild, and tended to increase in incidence and severity in the males in a dose-related manner; however, incidences were not reported. Cholesterol was the only serum end point that had values exceeding the reference range. Serum

cholesterol was significantly increased at 368 and 735 mg/kg/day in both sexes, but this change could be pituitary-related (see discussion of the 90-day study in Endocrine Effects).

Hepatic effects in the 90-day study included significantly increased relative liver weight (absolute weight not reported) and histopathological changes at ≥147 mg/kg/day in both sexes. The liver lesions included inflammation, hepatocellular alterations (characterized by spherical, brightly eosinophilic homogeneous inclusions), and hepatocellular necrosis. Liver lesions that were significantly increased included hepatocellular cytoplasmic alterations of minimal to mild severity in males at ≥147 mg/kg/day (incidences in the control to high dose groups were 1/10, 2/10, 1/10, 6/10, and 7/9) and females at 588 mg/kg/day (0/10, 2/10, 0/10, 1/10, and 7/9), and necrotic hepatocyte foci of minimal severity in both sexes at 588 mg/kg/day (1/10, 2/10, 1/10, 2/10, and 5/9 in males, and 0/10, 0/10, 0/10, 3/10, and 5/9 in females). Other statistically significant liver-associated effects included significantly increased serum AST levels (90–100% higher than controls) in males at ≥9 mg/kg/day and females at ≥37 mg/kg/day. Serum LDH levels were also reduced in males at ≥9 mg/kg/day, but the biological significance of a decrease in liver enzymes is unclear. Serum cholesterol values were significantly increased in males at ≥9 mg/kg/day and females at ≥37 mg/kg/day, but this change could be pituitary-related (see Endocrine Effects).

1,4-Dichlorobenzene. A single case study was located regarding hepatic effects in humans after oral exposure to 1,4-DCB. In this case report, the author describes a 3-year-old boy who had been playing with crystals containing 1,4-DCB for 4–5 days before being admitted to the hospital. On admission, the boy was jaundiced and his mucous membranes were pale. After a blood transfusion, the child gradually improved. It was unclear whether the boy actually ingested any of the 1,4-DCB (Hallowell 1959).

The acute hepatotoxicity and response of hepatic cytochrome P-450 in response to dosing with 1,4-DCB were evaluated in groups of male F344 rats (n=1/group) given one dose of 13–2,790 mg/kg body weight by corn oil gavage. Twenty-four hours after dosing, the animals were weighed and sacrificed. Serum was collected and analyzed for total bilirubin, cholesterol, AST, alanine aminotransferase (ALT), and alkaline phosphatase. The liver was weighed and slices examined histopathologically. Liver microsomes were prepared and assayed for P-450, in addition to liver protein determinations. 1,4-DCB did not produce liver necrosis at any dose. There was also no effect observed on serum levels of ALT and AST. Hepatic cytochrome P-450 levels were increased about 30% by 1,4-DCB beginning at 380 mg/kg and remaining elevated at all higher doses. No consistent pattern of change was found for indicators of

hepatobiliary damage, serum cholesterol, serum alkaline phosphatase, and total bilirubin (Allis et al. 1992).

The effects of 1,4-DCB were compared in male F344 rats given 0 (corn oil control), 25, 75, 150, and 300 mg/kg/day 1,4-DCB (n=6–8/group/time) by daily oral gavage 5 days/week for 1 week. Replicative DNA synthesis was studied using subcutaneously implanted osmotic pumps containing 5-bromo-2'-deoxyuridine (BrdU) to determine the hepatocyte labeling index. Livers were removed, weighed, and then immunostained. Morphological examination of the liver sections from all lobes was performed from control and 300 mg/kg group rats. 1,4-DCB treatment for 1 week did not produce morphological changes in the rat livers. 1,4-DCB produced significant dose-related increases in relative liver weight in the rats, which were also associated with mild centrilobular hypertrophy. At 300 mg/kg, relative liver weight was significantly increased. Significant dose-related increases in microsomal cytochrome P-450 content were observed in rats given 150 and 300 mg/kg 1,4-DCB for 1 week, with a significant dose-related induction of microsomal 7-pentoxyresorufin O-depentylase activity observed in rats given 75–300 mg/kg 1,4-DCB. The hepatocyte labeling index values were only increased in animals given 300 mg/kg 1,4-DCB (225% of controls) (Lake et al. 1997).

In a series of experiments, Eldridge et al. (1992) studied the acute hepatotoxic effects of 1,4-DCB and the role of cell proliferation in hepatotoxicity in $B6C3F_1$ mice and F344 rats. Mice and rats received a single dose of 1,4-DCB by gavage in corn oil of 600, 900, or 1,200 mg/kg/day. At 1, 2, 4, and 8 days after 1,4-DCB treatment, selected animals were injected intraperitoneally with BrdU 2 hours prior to sacrifice to monitor cell proliferation. Other groups of mice and rats were sacrificed 24 or 48 hours after dosing, blood was collected for liver enzyme analysis, and liver sections were collected for histopathology. In mice dosed with 600 mg/kg/day 1,4-DCB, liver weights were significantly increased 48 hours after dosing. Labeling index (LI), indicative of cell proliferation, peaked 24 hours after dosing in females and 48 hours in males. Activities of serum enzymes associated with liver damage (ALT, AST, LDH, sorbitol dehydrogenase) were not affected by 1,4-DCB. Twenty-four and 48 hours after administration of 1,4-DCB, the livers of males showed periportal hepatocytes with vacuolated cytoplasm and centrilobular hepatocytes with granulated basophilic cytoplasm; the severity of these changes was dose-related at 48 hours, but not at 24 hours. Similar but less pronounced effects were seen in females at 24 hours. In rats, liver weights were significantly increased at all time points after administration of 600 mg/kg/day 1,4-DCB. The LI peaked 24 hours after dosing and was still elevated after 48 hours. Necrosis was not observed in the livers of mice or rats after treatment with 1,4-DCB.

In pregnant CD rats administered 1,4-DCB in corn oil at doses of 250–1,000 mg/kg/day on Gd 6–15, no differences in maternal liver weight were noted (Giavini et al. 1986); however, hepatic effects have been reported in other oral studies in which 1,4-DCB has been administered to test animals by gavage (discussed below). These effects have ranged from temporary elevation of hepatic enzymes to hepatic degeneration and necrosis.

The effects of 1,4-DCB were compared in male B6C3F_1 mice given 0 (corn oil control), 300, and 600 mg/kg/day 1,4-DCB (n=6–8/group/time) by daily oral gavage 5 days/week for 1 week. Replicative DNA synthesis was studied using subcutaneously implanted osmotic pumps containing BrdU to assess the hepatocyte labeling index. Livers were removed, weighed, and immunostained. Morphological examination of the liver sections was performed for control and 600 mg/kg groups. Biochemical analysis of liver whole homogenates was performed. 1,4-DCB produced significant dose-related increases in relative liver weight, which were associated with marked centrilobular hypertrophy. Relative liver weights were increased for mice in both the 300 and 600 mg/kg groups at all time points, with minimal centrilobular hypertrophy observed in 600 mg/kg group mice. No other histological abnormalities were observed in the liver sections. Administration of 1,4-DCB also produced a sustained induction of microsomal cytochrome P-450 content and 7-pentoxyresorufin O-depentylase activity. Significant dose-related induction of microsomal cytochrome P-450 content was induced in mice given 600 but not 300 mg/kg 1,4-DCB. Microsomal 7-pentoxyresorufin O-depentylase activity was significantly induced in mouse liver microsomes at doses of 300 and 600 mg/kg 1,4-DCB. Western immunoblotting studies demonstrated that 1,4-DCB induced CYP2B isoenzyme(s) in mouse liver microsomes at 300 and 600 mg/kg 1,4-DCB. The hepatocyte labeling index values were also significantly increased in mice given 300 and 600 mg/kg 1,4-DCB (Lake et al. 1997).

In male B6C3F_1 mice, single doses of 600, 1,000, or 1,800 mg/kg/day 1,4-DCB administered by gavage in corn oil resulted in significantly elevated BrdU labeling of hepatocytes at the 1,000 and 1,800 mg/kg/day doses. In addition, single doses of 1,800 mg/kg resulted in a 4.5-fold increase in serum ALT activity and severe centrilobular hepatocyte swelling. In a companion time-course study, single doses of 1,800 mg/kg 1,4-DCB administered by gavage in corn oil resulted in significantly elevated BrdU labeling in hepatic samples on days 2, 3, and 4, but not days 1 or 7. ALT activity was significantly elevated in 1,4-DCB-treated mice on day 2 only. In all other aspects, hepatic toxicity was not evident in mice dosed with 1,800 mg/kg 1,4-DCB (Umemura et al. 1996).

1,4-DCB has been shown to produce disturbances in porphyrin metabolism after high-level/acute-duration exposure. Increased excretion of porphyrins, especially coproporphyrin and uroporphyrin, are considered to be indicators of liver damage. Administration of 1,4-DCB in liquid paraffin to male rats at gradually increasing doses, until a dose level of 770 mg/kg/day was maintained for 5 days, resulted in high porphyrin excretion (Rimington and Ziegler 1963). Mean peak values of urinary coproporphyrin increased to about 10–15-fold above levels in controls. A 37–100-fold increase in urinary uroporphyrin levels occurred; porphobilinogen levels increased 200–530-fold; and a 10-fold increase in δ-aminolevulinic acid (δ-ALA) levels was observed. In the liver itself, coproporphyrin levels were similar to controls, uroporphyrin levels were increased 46-fold, and protoporphyrin levels were increased 6-fold. These dramatic increases, which suggest severe damage to the liver, were not observed when 1,4-DCB was administered to rats at higher levels (850 mg/kg/day) in 1% cellofas (Rimington and Ziegler 1963) or at lower levels for a longer period of time in another study (Carlson 1977), as discussed below. Also, Trieff et al. (1991) have used animal data on porphyrogenicity from various chlorinated benzenes to perform a QSAR study allowing prediction of ambient water criteria.

Changes in other markers of liver function including cytochrome P-450 levels, and activities of some drug-metabolizing enzymes (aminopyrine N-demethylase and aniline hydroxylase) were investigated in rats treated with of 1,4-DCB by gavage at 250 mg/kg/day for up to 3 days (Ariyoshi et al. 1975). Activity of δ-ALA synthetase, an enzyme used in synthesis of the heme moiety found in cytochromes, was increased 42% by treatment with 1,4-DCB. However, the cytochrome P-450 content did not change, although the microsomal protein content of liver preparations was increased. The toxicological significance of these findings is not clear since δ-ALA synthetase activity did not correlate with cytochrome P-450 concentration.

Effects on hepatic enzyme activities were reported to have occurred in adult male rats that were given 1,4-DCB by gavage for 14 days (Carlson and Tardiff 1976). Significant decreases in hexobarbital sleeping time and a 6.5-fold increase in serum isocitrate dehydrogenase activity were observed after a 14-day treatment regimen at 650 mg/kg/day. In addition, even at considerably lower levels (20 or 40 mg/kg/day), increases were observed in the activities of hepatic microsomal xenobiotic metabolic systems including levels of glucuronyl transferase, and benzpyrene hydroxylase and O-ethyl-O-nitro-phenyl phenylphosphorothionate (EPN) detoxification to nitrophenol. In a 90-day study at the same dosage levels, significant increases were seen in EPN detoxification, benzpyrene hydroxylase, and azoreductase levels. The former two levels were still elevated at 30 days after the cessation of administration of the compound. Most increases were noted at 20 mg/kg/day and above as in the 14-day

studies; however, azoreductase levels were elevated even at 10 mg/kg/day (Carlson and Tardiff 1976). These observations are important because they demonstrate that hepatic effects occur at levels of 1,4-DCB that are far below those associated with severe histopathology.

The effects of 1,4-DCB were compared in male F344 rats given 0 (corn oil control), 25, 75, 150, and 300 mg/kg/day 1,4-DCB (n=6–8/group/time) by daily oral gavage 5 days/week for 4 and 13 weeks. Replicative DNA synthesis was studied using subcutaneously implanted osmotic pumps containing BrdU during study weeks 3–4 and 12–13. Livers were removed, weighed, and then immunostained. Morphological examination of the liver sections was performed from control and 300 mg/kg group rats in the 13-week exposure group. 1,4-DCB treatment produced a mild centrilobular hypertrophy seen in rats given 300 mg/kg 1,4-DCB for 13 weeks. No other histological abnormalities were observed in the liver sections. 1,4-DCB produced significant dose-related increases in relative liver weight in the rats, which were associated with mild centrilobular hypertrophy. At 300 mg/kg, relative liver weight was significantly increased. Significant increases in relative liver weight were observed in rats given 75 and 150 mg/kg 1,4-DCB for 4 weeks and 150 mg/kg 1,4-DCB for 13 weeks. Administration of 1,4-DCB also produced a sustained induction of microsomal cytochrome P-450 content and 7-pentoxyresorufin O-dependentylase activity. Significant dose-related increases in microsomal cytochrome P-450 content were observed in rats given 25–300 mg/kg 1,4-DCB for 4 weeks and 75–300 mg/kg 1,4-DCB for 13 weeks. A significant dose-related induction of microsomal 7-pentoxyresorufin O-dependentylase activity was observed in rats given 75–300 mg/kg 1,4-DCB for 4 weeks and 25–300 mg/kg 1,4-DCB for 13 weeks. Western immunoblotting studies demonstrated that 1,4-DCB induced CYP2B isoenzyme(s) in rat liver microsomes at 75 and 300 mg/kg 1,4-DCB (Lake et al. 1997).

Histopathological effects in the liver, including cloudy swelling and centrilobular necrosis, were observed after gavage administration of 1,4-DCB in rats (two per group) at 500 mg/kg/day for 4 weeks; similar results (cloudy swelling, focal caseous necrosis) were obtained in rabbits (five per group) given 92 doses of 1,000 mg/kg/day 1,4-DCB in olive oil over a 219-day period (Hollingsworth et al. 1956). The interpretation of this study is limited by the size of the test groups and the fact that observations in controls were not presented. Histopathological changes were also reported in a 13-week study in which rats received 1,4-DCB by gavage (NTP 1987). Doses of 1,200 or 1,500 mg/kg/day produced degeneration and necrosis of hepatocytes. Serum cholesterol levels were increased by doses of 600 mg/kg/day or more in male rats and by ≥900 mg/kg/day in female rats, while serum triglycerides and protein levels were reduced at doses of ≥300 mg/kg/day in male rats. Urinary porphyrins were increased in both sexes at ≥1,200 mg/kg/day. However, these increases were modest and considered by the authors

to indicate mild porphyrinuria rather than hepatic porphyria. Liver porphyrins were not increased at any dose. In a second 13-week study in the same laboratory, hepatic effects were not observed in rats at dosage levels up to 600 mg/kg/day (NTP 1987).

Similar hepatic effects were reported in two 13-week gavage studies in mice (NTP 1987). Hepatocellular degeneration was observed in both sexes at all doses (600–1,800 mg/kg/day). Serum cholesterol levels were increased in male mice at doses of 900 mg/kg/day or more, and serum protein and triglycerides were increased at doses of 1,500 mg/kg/day or more. These changes were thought by the authors to reflect the hepatic effects of this compound. Hepatic porphyria was not found in mice at any dose level in this study. Because hepatic effects were seen in mice in all dose groups in the first 13-week study, a second 13-week study was conducted at lower dosage levels. Hepatocellular cytomegaly was observed in mice at doses of 675 mg/kg/day and above. The lowest level at which hepatic effects were observed in mice was 600 mg/kg/day (in the first study).

Other intermediate-duration oral studies with 1,4-DCB have reported liver toxicity. In female rats dosed with 1,4-DCB by gavage for about 6 months, doses of 188 mg/kg/day and above resulted in increased liver weights. At 376 mg/kg/day, slight cirrhosis and focal necrosis of the liver were also observed (Hollingsworth et al. 1956). No effects on the liver were seen at a dose of 18.8 mg/kg/day.

The ability of 1,4-DCB to induce porphyria was investigated in female rats that were administered 1,4-DCB by gavage for up to 120 days (Carlson 1977). Slight but statistically significant increases in liver porphyrins were seen in all dosed rats (50–200 mg/kg/day) at 120 days. Urinary excretion of δ-ALA, porphobilinogen, or porphyrins was not increased over control levels. These results indicated that 1,4-DCB had only a slight potential for causing porphyria at these doses in female rats compared with the far more pronounced porphyrinogenic effects reported earlier in male rats that received 770 mg/kg/day for 5 days in a study by Rimington and Ziegler (1963). However, sex-related differences in susceptibility to 1,4-DCB's effects on these parameters cannot be ruled out in a comparison of these two studies.

The role of cell proliferation in liver toxicity induced by 1,4-DCB was examined in groups of mice (5–7 per sex per dose level) administered 0 (vehicle only), 300, or 600 mg/kg 1,4-DCB in corn oil by gavage 5 days/week for 13 weeks (Eldridge et al. 1992). The liver toxicity induced by 1,4-DCB was also examined in groups of female rats (5–7 per dose level) administered 0 (vehicle only) or 600 mg/kg 1,4-DCB in corn oil by gavage 5 days/week for 13 weeks. At various times during the study, mice were

implanted with osmotic pumps to deliver BrdU. Liver weights were significantly increased in high-dose male and female mice and in female rats throughout the 13-week study. Treated male mice showed a centrilobular pattern of labeled hepatocytes, whereas females were labeled throughout the lobules. At the lower-dose level, liver weight was increased in male and female mice at weeks 6 and 13. In a group of mice in which treatment with 600 mg/kg/day ceased after 5 weeks and the animals were allowed to recover for 1 week, liver weight returned to control values. The authors concluded that 1,4-DCB induced a mitogenic stimulation of cell proliferation in the liver rather than a regenerative response following cytotoxicity. This was evidenced by an increase in liver weight without increase in liver-associated plasma enzymes (Eldridge et al. 1992).

The effects of 1,4-DCB were determined in male B6C3F_1 mice given 0 (corn oil control), 300, and 600 mg/kg/day 1,4-DCB (n=6–8/group/time) by daily oral gavage 5 days/week for 4 and 13 weeks. Replicative DNA synthesis was studied using subcutaneously implanted osmotic pumps containing BrdU during study weeks 3–4 and 12–13. Livers were removed, weighed, and immunostained. Morphological examination of the livers was performed for control and 600 mg/kg group mice at 13 weeks. Biochemical analysis of liver whole homogenates was also performed. 1,4-DCB produced significant dose-related increases in relative liver weight in the mice, which were associated with marked centrilobular hypertrophy. Relative liver weights were increased for mice in both the 300 and 600 mg/kg groups at all time points. At 13 weeks, a marked centrilobular hypertrophy was observed in the 600 mg/kg group. No other histological abnormalities were observed in the liver. Administration of 1,4-DCB also produced a sustained induction of microsomal cytochrome P-450 content and 7-pentoxyresorufin O-depentylase activity. Significant dose-related induction of microsomal cytochrome P-450 content was induced in mice given 600 but not 300 mg/kg 1,4-DCB for treatments of 4 and 13 weeks. Microsomal 7-pentoxyresorufin O-depentylase activity was significantly induced in mouse liver microsomes at doses of 300 and 600 mg/kg 1,4-DCB. Western immunoblotting studies demonstrated that 1,4-DCB induced CYP2B isoenzyme(s) in mouse liver microsomes at 300 and 600 mg/kg 1,4-DCB. Hepatocyte labeling index values were significantly increased in mice given 300 and 600 mg/kg 1,4-DCB for 4 weeks (420 and 395% of controls, respectively) (Lake et al. 1997).

A 1-year study in dogs indicates that this species is more sensitive than rats or mice to hepatic effects of 1,4-DCB. Groups of five male and five female Beagle dogs were administered 1,4-DCB by capsule in dose levels of 0, 10, 50, or 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996). Liver effects occurred after 6 and 12 months at ≥50 mg/kg/day in both sexes as shown by changes in liver enzymes, increased liver weight, and/or histopathology. Serum levels of ALT, AST, GGT, and AP were measured

after 6 and 12 months. Statistically significant increases were found for serum AP in males at 50 mg/kg/day, and females at 50 and 75 mg/kg/day, at months 6 and 12 (330–761% higher than controls); ALT in females at 75 mg/kg/day and month 12 (253% higher than controls); and GGT in females at 75 mg/kg/day and months 6 and 12 (131–161% higher than controls). Serum albumin was significantly decreased in males at ≥50 mg/kg/day (months 6 and 12) and females at 75 mg/kg/day (month 6). Absolute and relative liver weights were significantly increased in both sexes at 50 and 75 mg/kg/day (except absolute liver weight in 50 mg/kg/day males). Hepatic lesions included hepatocellular hypertrophy in all males and females at 50 and 75 mg/kg/day (as well as one female at 10 mg/kg/day), hepatocellular pigment deposition at 50 and 75 mg/kg/day (two males and one female at each level), bile duct/ductule hyperplasia at 75 mg/kg/day (one male and one female), and hepatic portal inflammation at 50 and 75 mg/kg/day (periportal accumulation of neutrophils in an unspecified number of males). The 6- and 12-month increased serum AP levels in dogs (Naylor and Stout 1996) were used to derive intermediate- and chronic-duration oral MRLs of 0.07 mg/kg/day for 1,4-DCB.

Studies of the hepatic effects of chronic 1,4-DCB exposure are sparse. The toxicity of 1,4-DCB was evaluated in a group of seven rabbits administered 1,4-DCB in olive oil at a dose of 500 mg/kg/day a total of 263 times over a 367-day period. Slight changes in the liver (cloudy swelling and a few areas of focal caseous necrosis) were noted at sacrifice (Hollingsworth et al. 1956).

In the only study of lifetime oral exposure to 1,4-DCB in laboratory animals, groups of male and female F344 rats were administered 1,4-DCB by gavage in corn oil 5 days/week for 103 weeks at doses of 150 or 300 mg/kg/day (males) or 300 or 600 mg/kg/day (females). Groups of male and female $B6C3F_1$ mice were administered 1,4-DCB at doses of 300 or 600 mg/kg/day by gavage in corn oil, 5 days/week for 103 weeks. No hepatic effects were seen in rats; in mice, the incidence of hepatocellular degeneration was greatly increased in treated mice (in males: 0/50 control, 36/49 low-dose, 39/50 high-dose; in females 0/50 control, 8/48 low-dose, 36/50 high-dose). The primary degenerative change was cellular swelling with clearing or vacuolation of the cytoplasm. Individual hepatocytes had pyknotic or karyorrhectic nuclei and condensed eosinic cytoplasm. Some necrotic hepatocytes formed globular eosinophilic masses in the sinusoids (NTP 1987).

Renal Effects.

1,2-Dichlorobenzene. No studies were located regarding renal effects in humans after oral exposure to 1,2-DCB.

A single 1,500 mg/kg gavage dose of 1,2-DCB in peanut oil (a lethal level) caused accumulation of albuminous fluid and casts in the renal tubules of rats (number and gender not reported) (DuPont 1982). Sprague-Dawley rats (10/sex/level) that were administered 1,2-DCB in corn oil by gavage in doses of 300 mg/kg/day for 10 consecutive days or 400 mg/kg/day for 90 consecutive days (Robinson et al. 1991). In subchronic studies performed by NTP (1985), F344 rats and B6C3F_1 mice (10/sex/level/species) were administered 1,2-DCB in doses of 0, 30, 60, 125, 250, or 500 mg/kg/day in corn oil by gavage 5 days/week for 13 weeks. Histology examinations of the kidneys were limited to the 0 and ≥125 mg/kg/day dose groups in the rats and 0 and 500 mg/kg/day groups in the mice. Renal effects occurred only in the 500 mg/kg/day male rats; these included tubular degeneration (6/10 incidence compared to 0/10 in lower dose and control groups) and increased urine volume (57% higher than controls). There were no exposure-related increases in BUN in either species. In chronic studies performed by NTP (1985), there were no nonneoplastic tissue changes in the kidneys of male or female F344 rats (50/sex/level) exposed to 0, 60, or 120 mg/kg/day in corn oil by gavage for 5 days/week for 103weeks. In similarly-exposed B6C3F_1 mice (50/sex/level) exposure to 120 mg/kg/day, but not to 60 mg/kg/day, resulted in a significantly increased incidence of renal tubular regeneration (controls: 8/48; low dose: 12/50; high dose: 17/49) relative to controls. The incidence data for renal tubular regeneration in mice (NTP 1985) were used to derive a chronic-duration oral MRL of 0.3 mg/kg/day for 1,2-DCB. Renal end points other than histology were not assessed in the chronic studies.

1,3-Dichlorobenzene. No studies were located regarding renal effects in humans after oral exposure to 1,3-DCB.

No gross or histological changes were observed in the kidneys or urinary bladder in male or female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by gavage in doses of 735 mg/kg/day for 10 consecutive days or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995). Blood urea nitrogen (BUN) and kidney weight was measured in both studies, although only relative organ weights were reported. There was a statistically significant increase in relative kidney weight at ≥147 mg/kg/day in males and 735 mg/kg/day in females in the 90-day study, but this is not considered to be an adverse effect due to decreases in body weight gain and lack of changes in BUN and renal histology.

1,4-Dichlorobenzene. No studies were located regarding renal effects in humans after oral exposure to 1,4-DCB.

The role of cell proliferation in kidney toxicity induced by 1,4-DCB was examined in groups of male and female B6C3F_1 mice and F344 rats (Umemura et al. 1992). Mice were administered 300 or 600 mg/kg 1,4-DCB; in rats, males received 150 or 300 mg/kg 1,4-DCB while females received 300 or 600 mg/kg 1,4-DCB. All doses were administered by gavage in corn oil for 4 consecutive days. Cell proliferation was evaluated by means of immunohistochemical measurement of BrdU-labeled cells. In mice, kidney weights and cell proliferation in the kidney tubules were not altered by 1,4-DCB treatment; in rats, kidney weight was significantly increased in male rats at both dose levels, but was not affected in females. Cell proliferation was greatly increased in the proximal convoluted tubule from high-dose males. A lesser increase was seen in the proximal straight tubule from high-dose males; no increase was observed in the distal tubule from males or in any kidney region from treated female rats.

The effects of 1,4-DCB were compared in male F344 rats given 0 (corn oil control), 25, 75, 150, and 300 mg/kg/day 1,4-DCB (n=6–8/group/time) and male B6C3F_1 mice given 0 (corn oil control), 300, and 600 mg/kg/day 1,4-DCB (n=6–8/group/time) by daily oral gavage 5 days/week for 1 week. Replicative DNA synthesis was studied using subcutaneously implanted osmotic pumps containing 5-bromo-2'-deoxyuridine during study weeks 0–1, 3–4, and 12–13. After sacrifice, the kidneys were removed, weighed, and immunostained. In rats, significant increases in relative kidney weight were observed in those rats administered 150 and 300 mg/kg 1,4-DCB for 4 and 13 weeks. 1,4-DCB treatment produced significant increases in rat renal P1/P2 proximal tubule cell labeling index values at all time points. Significant increases were seen in the following groups: 75 mg/kg 1,4-DCB at 4 weeks (250% of controls); 150 mg/kg 1,4-DCB at 4 and 13 weeks (400 and 440% of controls, respectively); and 300 mg/kg 1,4-DCB at 1, 4, and 13 weeks (170, 475, and 775% of controls, respectively). A significant increase in rat P3 renal proximal tubule cell labeling index values was observed in 300 mg/kg 1,4-DCB group rats at weeks 4 (185% of controls) and 13 (485% of controls). In contrast, some reduction in rat P3 renal proximal tubule cell labeling index values was observed in 75–300 mg/kg 1,4-DCB group rats at 1 week. In contrast, 1,4-DCB treatment produced little effect on mouse renal P1/P2 proximal tubule cell labeling index values at all time points tested. No significant increase was seen in 300 or 600 mg/kg 1,4-DCB groups for 1 and 13 weeks, but significant increases were seen at 4 weeks (205 and 170% of controls, respectively). Neither 300 nor 600 mg/kg 1,4-DCB for 1, 4, or 13 weeks had much effect on mouse P3 renal proximal tubule cell labeling index values (Lake et al. 1997).

In a study that examined the role of the protein $\alpha_{2\mu}$-globulin in 1,4-DCB-induced nephrotoxicity in male rats, NCI-Black-Reiter (NBR) rats, known not to synthesize the hepatic form of the $\alpha_{2\mu}$-globulin, were administered 500 mg/kg/day 1,4-DCB by gavage in corn oil for 4 consecutive days. Positive controls

consisted of F344 male rats treated with lindane; the results were also compared with those obtained in a group of female F344 rats treated with lindane. End points examined consisted of kidney lesions and protein droplet evaluation. $\alpha_{2\mu}$-Globulin was detected in kidney sections from male F344 rats, but not in male NBR or female F344 rats. No lesions or hyaline droplets were detected in treated or control male NBR and female F344 rats (Dietrich and Swenberg 1991).

Renal tubular degeneration has been observed in male but not female F344 rats in two 13-week gavage studies (NTP 1987). These effects were severe in male rats receiving ≥300 mg/kg/day in the first study, but in the second study, only slight changes were seen at 300 mg/kg/day, while moderate tubular degeneration was present at 600 mg/kg/day. Renal effects reported in another intermediate-duration gavage study in rats included increased renal weights at doses of ≥188 mg/kg/day (Hollingsworth et al. 1956). Renal effects were not observed in mice in either of two 13-week gavage studies using dosage regimens of 600–1,800 and 84.4–900 mg/kg/day (NTP 1987).

In a study designed to investigate the mechanism of renal toxicity for 1,4-DCB reported in the NTP (1987) studies, 1,4-DCB administered by gavage to male F344 rats at 7 daily doses of 120 or 300 mg/kg/day significantly increased the level of protein droplet formation in the kidneys of males but not females (Charbonneau et al. 1987). Administration of a single dose of ^{14}C-1,4-DCB by gavage at 500 mg/kg gave similar results. An analysis of the renal tissue of animals administered radio-labeled 1,4-DCB indicated that it was reversibly associated with the protein $\alpha_{2\mu}$-globulin. In a study designed to correspond to the experimental conditions of the 13-week NTP (1987) study in rats, 1,4-DCB was administered to F344 rats by gavage at 75–600 mg/kg/day for 13 weeks; interim sacrifices were performed at 4 weeks (Bomhard et al. 1988). At 4 weeks, females had no structural damage to the kidneys, while males experienced damage at the corticomedullary junction at doses of 150 mg/kg or more; damage consisted of dilated tubules with granular and crystalline structures, hyaline droplets, and desquamated epithelia. At all dose levels in the males, hyaline bodies were seen in the proximal tubule epithelial cells. At 13 weeks, males exhibited an increase urinary excretion of LDH and of epithelial cells over the entire dose range tested. These changes did not always appear to be dose-related. No signs of structural damage were seen in the females' kidneys. In males, a dose-dependent incidence of hyaline droplets in the cortical tubular epithelium was seen at 75 mg/kg/day and above. At ≥150 mg/kg/day, single-cell necrosis was observed, and at 300 and 600 mg/kg/day, epithelial desquamation of longer parts of the tubules were occasionally seen.

In the only available study of chronic-duration oral exposure to 1,4-DCB, renal effects were observed to occur preferentially in male rats. Male F344 rats exposed to 1,4-DCB at 150 and 300 mg/kg/day by gavage for 2 years exhibited the following effects with greater severity and in greater numbers: nephropathy, epithelial hyperplasia of the renal pelvis, mineralization of the collecting tubules in the renal medulla, and focal hyperplasia of renal tubular epithelium (NTP 1987). There was also increased incidence of nephropathy in female rats dosed with 1,4-DCB at 300 and 600 mg/kg/day, but there was minimal hyperplasia of the renal pelvis or tubules. Administration of 1,4-DCB at 300 and 600 mg/kg/day for 2 years also increased the incidence of nephropathy in male $B6C3F_1$ mice. Renal tubular degeneration was noted in female mice, but these changes occurred at a lower frequency and were qualitatively different from those in male rats (NTP 1987).

In a study with dogs, groups of five male and five female Beagles were administered 1,4-DCB by capsule in dose levels of 0, 10, 50, or 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996). Histopathological changes were observed in the kidneys that included collecting duct epithelial vacuolation in one male at 75 mg/kg/day, and in females at all dose levels (one at 10 mg/kg/day, one at 50 mg/kg/day, and two at 75 mg/kg/day). This renal lesion was considered to be a possible effect of treatment at ≥50 mg/kg/day where it was accompanied by increased relative kidney weight (50 mg/kg/day females) and gross observed renal discoloration (two females at 75 mg/kg/day). No gross or histological changes were found in the urinary bladder.

Endocrine Effects.

1,2-Dichlorobenzene. No studies were located regarding endocrine effects in humans after oral exposure to 1,2-DCB.

No gross or histological changes were observed in the adrenal or pancreas of Sprague-Dawley rats that were administered 1,2-DCB in corn oil by gavage in a dose of 300 mg/kg/day for 10 consecutive days, or in the adrenal (pancreas not examined) in rats similarly exposed to 400 mg/kg/day for 90 consecutive days (Robinson et al. 1991). No gross or histological changes were observed in the adrenal, pancreas, thyroid, parathyroid, or pituitary of F344 rats or $B6C3F_1$ mice that were treated with 1,2-DCB in corn oil by gavage in doses ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985), or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985).

1,3-Dichlorobenzene. No studies were located regarding endocrine effects in humans after oral exposure to 1,3-DCB.

Gross and histological examinations of adrenals, pancreas, pituitary, thyroid, parathyroids, and gonads were performed in groups of 10 male and 10 female Sprague-Dawley rats that were exposed to 1,3-DCB in oil by daily gavage, in doses of 0 or 735 mg/kg/day for 10 consecutive days or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995). The 90-day study additionally included examinations of thyroid and pituitary at lower dose levels of 9, 37, and 147 mg/kg/day. No compound-related endocrine effects were observed in the 10-day study. As discussed below, the 90-day study found histological effects in the thyroid at ≥9 mg/kg/day and the pituitary at ≥147 mg/kg/day. The only other tissue with histological changes in the 90-day study was the liver (see Hepatic Effects).

Inflammatory and degenerative lesions in the McCauley et al. (1995) 90-day study were graded on a relative scale from one to four depending on severity (minimal, mild, moderate, or marked). In the thyroid, colloidal density in the follicular cells was significantly ($p \leq 0.05$) increased in male rats at ≥9 mg/kg/day and female rats at ≥37 mg/kg/day. The incidences of this lesion in the 0, 9, 37, 147, and 588 mg/kg/day dose groups were 2/10, 8/10, 10/10, 8/9, and 8/8 in males and 1/10, 5/10, 8/10, 8/10, and 8/9 in females. Depletion of colloid density in the thyroid was characterized by decreased follicular size with scant colloid and follicles lined by cells that were cuboidal to columnar. The severity of the colloid density depletion generally ranged from mild to moderate, increased with dose level, and was greater in males than females. For example, in the 147 and 588 mg/kg/day groups, severity was classified as moderate in males and mild for the females. Incidences of male rats with thyroid colloidal density depletion of moderate or marked severity were significantly increased at ≥147 mg/kg/day (0/10, 0/10, 2/10, 5/9, and 6/8). The lowest tested dose, 9 mg/kg/day, is considered to be a minimal LOAEL because the morphological alterations (reduced colloidal density in follicles) are unlikely to be associated with functional changes in the thyroid. The pituitary effect was cytoplasmic vacuolization in the *pars distalis* and only found in the male rats. Incidences of this lesion were significantly ($p \leq 0.05$) increased in males at ≥147 mg/kg/day (2/10, 6/10, 6/10, 10/10, and 7/7); incidences in the 9 and 37 mg/kg/day groups were marginally increased ($p = 0.085$). The vacuoles were variably sized, irregularly shaped, and often poorly defined, and severity (number of cells containing vacuoles) ranged from minimal to mild. The severity of the lesions generally increased with increasing dose level, and incidences of male rats with pituitary cytoplasmic vacuolization of moderate or marked severity were significantly increased at 588 mg/kg/day (1/10, 0/10, 2/10, 3/9, and 7/7). The pituitary lesion was reported to be similar to “castration cells” found in gonadectomized rats, and considered to be an indicator of gonadal deficiency. No compound-related

pituitary lesions were observed in female rats. The incidences of pituitary lesions in male rats (McCauley et al. 1995) were used to derive an intermediate-duration oral MRL of 0.02 mg/kg/day for 1,3-DCB. Other effects in this 90-day study included significant increases in serum cholesterol in males at ≥9 mg/kg/day and females at ≥37 mg/kg/day, and serum calcium in both sexes at ≥37 mg/kg/day. The study authors suggested that these serum chemistry changes might reflect a disruption of hormonal feedback mechanisms, or target organ effects on the pituitary, hypothalamus, and/or other endocrine organs.

1,4-Dichlorobenzene. No studies were located regarding endocrine effects in humans after oral exposure to 1,4-DCB.

In a series of dose range-finding studies, groups of F344 rats were administered 1,4-DCB at concentrations ranging from 37.5 to 1,500 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks. At sacrifice, animals were examined grossly and major tissues were examined histologically. No endocrine organs were affected in any of the 1,4-DCB-treated rats. In parallel studies with $B6C3F_1$ mice, no compound-related endocrine effects were observed after administration of 1,4-DCB at concentrations ranging from 84.4 to 1,800 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks (NTP 1987).

In the only study of lifetime oral exposure to 1,4-DCB in laboratory animals (NTP 1987), groups of male and female F344 rats were administered 1,4-DCB by gavage in corn oil, 5 days/week for 103 weeks at doses of 150 or 300 mg/kg/day (males) or 300 or 600 mg/kg/day (females). Groups of male and female $B6C3F_1$ mice were administered 1,4-DCB at doses of 300 or 600 mg/kg/day by gavage in corn oil, 5 days/week for 103 weeks. In the F344 rats, an increased incidence of parathyroid hyperplasia was observed in males (4/42 controls, 13/42 low-dose, 20/38 high-dose), while no effect was seen in females. In mice, the incidence of thyroid follicular cell hyperplasia increased with dose in males (1/47 control, 4/48 low-dose, 10/47 high-dose), but not in females. The incidence of adrenal medullary hyperplasia and focal hyperplasia of the adrenal gland capsule also increased with dose in males (controls, 11/47; low-dose, 21/48; high-dose, 28/49).

No gross or histological changes were found in the adrenal, thyroid, parathyroid, pancreas, or pituitary glands of Beagle dogs (5/sex/level) that were administered 1,4-DCB by capsule in doses as high as 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996).

Dermal Effects.

1,2-Dichlorobenzene. No studies were located regarding dermal effects in humans after oral exposure to 1,2-DCB.

No gross or histological changes were observed in the skin of Sprague-Dawley or F344 rats that were administered 1,2-DCB in corn oil by gavage in doses of 300 mg/kg/day for 10 consecutive days (Robinson et al. 1991), ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985), or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985). Additionally, there were no gross or histological effects in the skin of $B6C3F_1$ mice that were similarly treated with ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985) or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985).

1,3-Dichlorobenzene. No studies were located regarding dermal effects in humans after oral exposure to 1,3-DCB.

No gross or histological changes were observed in the skin in male or female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by gavage in doses of 735 mg/kg/day for 10 consecutive days or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995).

1,4-Dichlorobenzene. A 19-year-old black woman who had been eating 4–5 moth pellets made of 1,4-DCB daily for 2.5 years developed symmetrical, well demarcated areas of increased pigmentation in a bizarre configuration over various parts of her body. After she discontinued this practice, the skin discolorations gradually disappeared over the next 4 months (Frank and Cohen 1961).

In laboratory animals, groups of F344 rats were administered 1,4-DCB at concentrations ranging from 37.5 to 1,500 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks. No dermal effects were noted in any of the 1,4-DCB-treated rats. In parallel studies with $B6C3F_1$ mice, no compound-related dermal effects were observed after administration of 1,4-DCB at concentrations ranging from 84.4 to 1,800 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks (NTP 1987).

In the only study of lifetime oral exposure to 1,4-DCB in laboratory animals (NTP 1987), groups of male and female F344 rats were administered 1,4-DCB by gavage in corn oil, 5 days/week for 103 weeks at doses of 150 or 300 mg/kg/day (males) or 300 or 600 mg/kg/day (females). Groups of male and female $B6C3F_1$ mice were administered 1,4-DCB at doses of 300 or 600 mg/kg/day by gavage in corn oil,

5 days/week for 103 weeks. No dermal effects have been reported in rats or mice at any of the studied doses.

No gross or histological changes were found in the skin of Beagle dogs (5/sex/level) that were administered 1,4-DCB by capsule in doses as high as 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996).

Ocular Effects.

1,2-Dichlorobenzene. No studies were located regarding ocular effects in humans after oral exposure to 1,2-DCB. Opthalmoscopic examinations showed no effects in Sprague-Dawley rats that were dosed with 400 mg/kg/day of 1,2-DCB in corn oil by gavage for 90 consecutive days (Robinson et al. 1991). No gross or histological changes were observed in eyes of F344 rats or B6C3F$_1$ mice that were similarly exposed to ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985) or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985).

1,3-Dichlorobenzene. No studies were located regarding ocular effects in humans or animals after oral exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding the ocular effects in humans after oral exposure to 1,4-DCB.

In a series of intermediate-duration studies, groups of F344 rats were administered 1,4-DCB at concentrations ranging from 37.5 to 1,500 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks. Ocular discharge was noted prior to death in males dosed with 1,200 mg/kg and in all rats exposed to 1,500 mg/kg. In parallel studies with B6C3F$_1$ mice, no compound-related ocular effects were observed after administration of 1,4-DCB at concentrations ranging from 84.4 to 1,800 mg/kg/day by gavage in corn oil 5 days/week for 13 weeks (NTP 1987).

The ocular effects of oral administration of 1,4-DCB were examined in groups of white (strain not reported) female rats and male and female rabbits. Rats received 1,4-DCB in olive oil at doses of 18.8–376 mg/kg/day, 5 days/week for 192 days; rabbits received 1,4-DCB in olive oil at a dose of 1,000 mg/kg/day for 219 days. Under the study conditions, administration of 1,4-DCB did not produce cataracts in either species (Hollingsworth et al. 1956).

In chronic-duration toxicity studies in laboratory animals, Hollingsworth et al. (1956) found no evidence of cataract formation in rabbits administered a total of 263 doses of 500 mg/kg/day 1,4-DCB in olive oil over a 367-day period.

In two lifetime oral exposure studies (NTP 1987), groups of male and female F344 rats were administered 1,4-DCB by gavage in corn oil, 5 days/week for 103 weeks at doses of 150 or 300 mg/kg/day (males) or 300 or 600 mg/kg/day (females); groups of male and female B6C3F_1 mice were administered 1,4-DCB at doses of 300 or 600 mg/kg/day by gavage in corn oil, 5 days/week for 103 weeks. In both species, no ocular effects were noted at any of the studied doses.

Opthalmoscopic examination showed no ocular effects in Beagle dogs (5/sex/level) that were administered 1,4-DCB by capsule in doses as high as 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996).

Body Weight Effects.

1,2-Dichlorobenzene. No studies were located regarding body weight effects in humans after oral exposure to 1,2-DCB.

Gavage exposure to 1,2-DCB in oil has adversely affected body weight gain in rodent at doses that also caused other signs of toxicity. Decreases in body weight gain in the range of 10–20% were observed in rats exposed to 300 mg/kg/day for 10 consecutive days (Robinson et al. 1991), 400 mg/kg/day for 90 consecutive days (Robinson et al. 1991), 1,000 mg/kg/day for 14 consecutive days (NTP 1985), and 500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985), as well as in mice exposed to 500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985).

1,3-Dichlorobenzene. No studies were located regarding body weight effects in humans after oral exposure to 1,3-DCB.

Body weight was measured in groups of 10 male and 10 female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by daily gavage, in doses of 0, 37, 147, 368, or 735 mg/kg/day for 10 consecutive days, or 9, 37, 147, or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995). Decreases in body weight gain occurred in both sexes at the high dose in both studies. In the 10-day study, final body

weights at 735 mg/kg/day were 20 and 13% lower than controls in males and females, respectively. The weight loss was progressive throughout the exposure period and, in males, accompanied by significantly reduced food consumption (12%, normalized by body weight). In the 90-day study, final body weights at 588 mg/kg/day were 24 and 10% lower than controls in males and females, respectively. The weight loss was progressive throughout the exposure period, and occurred despite increased food and water consumption.

1,4-Dichlorobenzene. No studies were located regarding body weight effects in humans after oral exposure to 1,4-DCB.

The effects of acute exposure to 1,4-DCB on body weight were examined in female Wistar rats given 1,4-DCB suspended in 2% tragacanth gum solution (a suspending agent obtained from the dried gummy exudation of *Astragalus gummifer*) at a dose of 250 mg/kg/day for 3 days. Under these conditions, no effects on body weight were seen (Ariyoshi et al. 1975). Male and female mice and female rats dosed once with 600 mg/kg/day 1,4-DCB also showed no discernible changes in body weight (Eldridge et al. 1992). Male rats administered 770 mg/kg/day of 1,4-DCB once a day for 5 days showed no changes in body weight (Rimington and Ziegler 1963). Pregnant CD rats that were administered 250–1,000 mg/kg/day 1,4-DCB in corn oil on Gd 6–15 experienced a reversible loss in maternal body weight (Giavini et al. 1986).

Body weight changes were observed in three studies in rats and mice (NTP 1987). In the first, both sexes of mice and female rats dosed at concentrations up to 1,000 mg/kg/day for 14 days by gavage demonstrated no changes in body weight during the test period. Male rats dosed at 500 mg/kg/day also showed no changes in body weight; however, a 7–12% decrease in body weight was noted in the 1,000 mg/kg/day dose group. In the second study (same route and duration as the first), male mice experienced a 13.3% decrease in body weight at the 250 mg/kg/day dose and a 14.7% decrease in body weight at the 2,000 mg/kg/day dose; however, results of intermediate doses demonstrated that there was no observable dose-response relationship for body weight changes. Neither male nor female rats dosed with 500 mg/kg/day showed any effects on body weights; however, a dose of 1,000 mg/kg/day resulted in a 13.5% decrease in weight for males and a 16.7% decrease in females. In the third study, male rats gavaged with 0, 25, 75, or 150 mg/kg of 1,4-DCB in corn oil for 7 days showed no changes in body weight; however, rats dosed at 300 mg/kg showed an approximately 10% decrease in body weight gain (Lake et al. 1997). The same study in male mice dosed with 0, 300, or 600 mg/kg of 1,4-DCB in corn oil for 7 days showed no changes in body weight at any dose level (Lake et al. 1997).

In intermediate-duration studies, no compound-related effects on weight gain were noted in albino or F344 rats administered 1,4-DCB by gavage in corn oil at doses up to 600 mg/kg/day, 7 days/week for 13 weeks (Bomhard et al. 1988; Carlson and Tardiff 1976). Male rats gavaged with 0 or 25 mg/kg of 1,4-DCB in corn oil for 7 days showed no changes in body weight; however, rats dosed at 75, 150, or 300 mg/kg showed an approximately 10% decrease in body weight gain (Lake et al. 1997). The same study in male mice dosed with 0, 300, or 600 mg/kg of 1,4-DCB in corn oil for 7 days showed no changes in body weight at any dose level (Lake et al. 1997). Male and female mice and female rats dosed with concentrations of 600 mg/kg/day 1,4-DCB 5 days/week for 13 weeks also showed no discernible changes in body weight (Eldridge et al. 1992). In a series of dose range-finding studies, groups of F344 rats were administered 1,4-DCB at concentrations ranging from 37.5 to 1,500 mg/kg/day by gavage in corn oil, 5 days/week for 13 weeks (NTP 1987). In the first of these studies, there were no treatment-related effects on body weight at doses up to 600 mg/kg/day. In the second study, final body weight was decreased by 11% in low-dose males (300 mg/kg/day) relative to controls; in high-dose males (1,500 mg/kg/day), the reduction was 32%. The effect was less marked in females (6% reduction at 900 mg/kg/day; 11% reduction at 1,200 mg/kg/day). In parallel studies with $B6C3F_1$ mice, no compound-related effects on body weight were observed after administration of 1,4-DCB at concentrations up to 900 mg/kg/day; however, in the second study, final body weight was reduced in all males receiving 1,4-DCB (11.4% at 1,500 mg/kg/day to 13.9% at 600 mg/kg/day) and in females at 600 mg/kg/day (10.3%) (NTP 1987).

In two lifetime oral exposure studies, groups of male and female F344 rats and $B6C3F_1$ mice were administered 1,4-DCB by gavage in corn oil, 5 days/week for 103 weeks. Fischer 344 rats were administered 1,4-DCB at doses of 150 or 300 mg/kg/day (males) or 300 or 600 mg/kg/day (females); mice were administered 1,4-DCB at doses of 300 or 600 mg/kg/day (NTP 1987). In mice, no effects on body weight attributable to treatment with 1,4-DCB were observed at doses up to 600 mg/kg/day. In rats, body weight gain was depressed by 12.5% in high-dose males (300 mg/kg/day) and by 12.4% in high-dose females (600 mg/kg/day) relative to vehicle controls.

There were no adverse body weight changes in Beagle dogs (5/sex/level) that were administered 1,4-DCB by capsule in doses as high as 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996).

3.2.2.3 Immunological and Lymphoreticular Effects

1,2-Dichlorobenzene. No studies were located regarding immunological or lymphoreticular effects in humans after oral exposure to 1,2-DCB.

Immunological function has not been assessed in animals orally exposed to 1,2-DCB. No gross or histological changes were observed in the spleen, thymus, or lymph nodes of male or female Sprague-Dawley rats that were administered 1,2-DCB in corn oil by gavage in doses of 300 mg/kg/day for 10 consecutive days or 400 mg/kg/day for 90 consecutive days (Robinson et al. 1991). Gross and histological examinations of lymph nodes, spleen, thymus, and bone marrow were performed in F344 rats and $B6C3F_1$ mice that were exposed to 1,2-DCB in corn oil by gavage 5 days/week in doses ≤500 mg/kg/day for 13 weeks or ≤120 mg/kg/day for 103 weeks (NTP 1985). The only changes in these tissues occurred at 500 mg/kg/day in the 13-week study; effects included lymphoid depletion in the thymus (4/10 male rats, 2/10 male mice, 2/10 female mice) and spleen (4/10 male mice, 2/10 female mice).

1,3-Dichlorobenzene. No studies were located regarding immunological or lymphoreticular effects in humans after oral exposure to 1,3-DCB.

Immunological function has not been assessed in animals orally exposed to 1,3-DCB. No gross or histological changes were observed in the spleen, thymus, or mandibular and mesenteric lymph nodes of male or female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by gavage in doses of 735 mg/kg/day for 10 consecutive days, or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995). Spleen and thymus weight was measured in both studies, although only relative organ weights were reported. In the 10-day study, relative spleen weight was significantly decreased in females at ≥368 mg/kg/day and males at 735 mg/kg/day, and relative thymus weight was significantly decreased in both sexes at 735 mg/kg/day. These changes are not considered adverse because body weight gain was decreased and they were not observed after 90 days or accompanied by histological alterations.

1,4-Dichlorobenzene. No studies were located regarding immunological effects in humans after oral exposure to 1,4-DCB. Symmetrical lesions with a bizarre pattern of skin pigmentation over most of her body were reported in the case study of a 19-year-old black woman who ingested 4–5 moth pellets of 1,4-DCB per day for a 2.5-year period (Frank and Cohen 1961). The lesion disappeared 4 months after

cessation. The described lesions may have been the result an immunological response to 1,4-DCB. However, this possibility was not addressed by the authors.

Groups of F344 rats were administered 1,4-DCB at concentrations ranging from 300 to 1,500 mg/kg/day by gavage in corn oil, 5 days/week for 13 weeks (NTP 1987). Treatment-related immunological and lymphoreticular effects noted in the study included hypoplasia of the bone marrow and lymphoid depletion of the spleen and thymus in males and females at doses of 1,200 mg/kg/day and above. In parallel studies with $B6C3F_1$ mice administered 1,4-DCB at concentrations ranging from 300 to 1,500 mg/kg/day, lymphoid necrosis in the thymus, lymphoid depletion in the spleen, and hematopoietic hypoplasia of the spleen and bone marrow were noted in both males and females at doses of 1,500 mg/kg/day and above (NTP 1987).

Minimal lymphoreticular changes were noted in a chronic-duration study (NTP 1987). Male rats administered doses of 150 or 300 mg/kg/day and female rats given 300 or 600 mg/kg/day of 1,4-DCB by gavage 5 days/week for 2 years showed no discernible changes in the lymphoreticular system; however, mice dosed in a similar fashion and at a dose of 600 mg/kg/day showed an increased incidence of lymph node hyperplasia.

No gross or histological changes were found in spleen, thymus, or lymph nodes of Beagle dogs (5/sex/level) that were administered 1,4-DCB by capsule in doses as high as 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996).

3.2.2.4 Neurological Effects

1,2-Dichlorobenzene. No studies were located regarding neurological effects in humans after oral exposure to 1,2-DCB.

Neurobehavioral function has not been assessed in animals orally exposed to 1,2-DCB. Ataxia and clonic contractions were observed in a small group of rats (three males) administered 1,2-DCB in liquid paraffin by gavage in a porphyrinogenic dose regimen of 455 mg/kg/day for 15 consecutive days (Rimington and Ziegler 1963). No clinical signs of neurotoxicity or histological changes in the brain were found in Sprague-Dawley or F344 rats that were administered 1,2-DCB in corn oil by gavage in doses of 300 mg/kg/day for 10 consecutive days (Robinson et al. 1991), 400 mg/kg/day for 90 consecutive days (Robinson et al. 1991), ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985), or ≤120 mg/kg/day,

5 days/week for 103 weeks (NTP 1985). The 10-day rat study also found no histological changes in sciatic nerve tissue, and the 90-day rat study also found no changes in absolute or relative brain weight (Robinson et al. 1991). Additionally, there were no signs of neurotoxicity or histological effects in the brain of $B6C3F_1$ mice that were gavaged with ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985) or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985).

1,3-Dichlorobenzene. No studies were located regarding immunological effects in humans after oral exposure to 1,3-DCB.

Neurobehavioral function has not been assessed animals orally exposed to 1,3-DCB. No clinical signs of neurotoxicity, or histological changes in the nervous system (brain or sciatic nerve), occurred in male or female Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by gavage in doses of 735 mg/kg/day for 10 consecutive days, or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995).

1,4-Dichlorobenzene. Two case studies have reported neurological effects in humans exposed to 1,4-DCB via ingestion have been reported in two case studies. A 21-year-old pregnant woman developed pica (a craving for unnatural substances) for 1,4-DCB toilet bowl deodorizer blocks, which she consumed at the rate of 1–2/week throughout pregnancy (Campbell and Davidson 1970). Reported neurological effects included fatigue, dizziness, and mild anorexia. These effects, however, are common general symptoms that occur in many women during normal pregnancy. A 19-year-old black woman who ingested 4–5 pellets of 1,4-DCB daily for about 2.5 years developed tremors and unsteadiness after she stopped eating this chemical. However, in the opinion of the neurologist who evaluated the woman in this case report, the effects were considered to be psychological rather than the physiological effects of withdrawal from 1,4-DCB (Frank and Cohen 1961).

Two studies in laboratory animals indicate that oral exposure to 1,4-DCB may result in adverse neurological effects. In a study performed by Rimington and Ziegler (1963), three male albino rats were administered daily doses of 1,4-DCB in liquid paraffin at gradually increasing doses until a dose was reached (770 mg/kg/day), which resulted in high porphyrin excretion with very few fatalities; this dose was given for 5 days. Clinical symptoms associated with highly porphyric rats included extreme weakness, ataxia, clonic contractions, and slight tremors (a rarity). One rat receiving 1,4-DCB developed left-sided hemiparesis. In F344 rats administered 1,4-DCB by gavage in corn oil 5 days/week for 13 weeks, tremors and poor motor response were observed in males at 1,200 mg/kg/day and above, and in

both sexes at 1,500 mg/kg/day. However, administration of 1,4-DCB had no effect on brain weight or on the microscopical appearance of the brain, sciatic nerve, or spinal cord (NTP 1987).

In a chronic-duration study (NTP 1987), no neurological effects were noted either in rats dosed with 300 mg/kg/day of 1,4-DCB, 5 days/week for 2 years, or in mice dosed with 600 mg/kg/day, 5 days/week for 2 years.

No gross or histological changes were found in the brain, spinal cord (three levels), or peripheral or optic nerves of Beagle dogs (5/sex/level) that were administered 1,4-DCB by capsule in doses as high as 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996).

3.2.2.5 Reproductive Effects

1,2-Dichlorobenzene. No studies were located regarding reproductive effects in humans after oral exposure to 1,2-DCB.

Reproductive function has not been assessed in animals orally exposed to 1,2-DCB. No gross or histological changes were observed in the testes, seminal vesicles, prostate, or ovaries of Sprague-Dawley rats that were administered 1,2-DCB in corn oil by gavage in a dose of 300 mg/kg/day for 10 consecutive days (Robinson et al. 1991). There were no changes in testis or ovary weight (absolute or relative) or histology in Sprague-Dawley rats that were similarly exposed to 400 mg/kg/day for 90 consecutive days (Robinson et al. 1991). Additionally, no gross or histological changes occurred in reproductive tissues of male (prostate, testes) or female (ovaries, uterus) F344 rats and B6C3F_1 mice that were similarly exposed to ≤500 mg/kg/day, 5 days/week for 13 weeks (NTP 1985) or ≤120 mg/kg/day, 5 days/week for 103 weeks (NTP 1985).

1,3-Dichlorobenzene. No studies were located regarding reproductive effects in humans after oral exposure to 1,3-DCB.

Reproductive function has not been assessed in animals orally exposed to 1,3-DCB. No histological changes occurred in male or female reproductive tissues (testes, seminal vesicles, prostate, preputial gland, clitoral gland, ovaries, or mammary gland).of Sprague-Dawley rats that were exposed to 1,3-DCB in corn oil by gavage in doses of 735 mg/kg/day for 10 consecutive days or 588 mg/kg/day for 90 consecutive days (McCauley et al. 1995). Testis and ovary weight was measured in both studies,

although only relative organ weights were reported. There was a statistically significant but small decrease (10.6% less than controls) in relative testes weight at 735 mg/kg/day in the 10-day study, but this is not considered to be an adverse effect because the magnitude of change was small, body weight gain was decreased, and there were no accompanying testicular histological alterations.

1,4-Dichlorobenzene. No studies were located regarding reproductive effects in humans after oral exposure to 1,4-DCB.

1,4-DCB was administered to female CD rats by gavage in corn oil on Gd 6–15 in a developmental toxicity study (Giavini et al. 1986). Doses up to 1,000 mg/kg/day had no adverse effect on the mean number of corpora lutea, mean number of implantations, mean percentage of pre- or postimplantation losses, or mean percentage of dams with resorptions (Giavini et al. 1986). In another developmental toxicity study of 1,4-DCB, female Wistar rats were exposed to a reported estimated dietary dose of 2 mg/kg/day from gestation day (Gd) 1 to postnatal day (Pnd) 21 for a total of 42 days (Makita 2005). There were no exposure-related effects on fertility, litter size, or sex ratio, and examinations of the pups at 6 weeks of age showed no changes in serum levels of reproductive hormones (leutinizing hormone [LH] and follicle stimulating hormone [FSH] in both sexes, testosterone in males) or weight or histology of reproductive tissues (testes, epididymides, prostate, seminal vesicles, ovaries, and uterus).

Intermediate- and chronic-duration toxicity studies were conducted in which F344/N and B6C3F_1 mice were treated with 1,4-DCB in corn oil by gavage 5 days/week (NTP 1987). No gross or histological changes were observed in reproductive tissues (testis, ovary, uterus, or mammary gland) of rats exposed to ≤1,500 mg/kg/day for 13 weeks or ≤300 mg/kg/day for 103 weeks, or mice exposed to ≤1,800 mg/kg/day for 13 weeks or ≤600 mg/kg/day for 103 weeks. No gross or histological changes were found in the testes, ovaries, or uterus of Beagle dogs that were administered 1,4-DCB by capsule in doses as high as 75 mg/kg/day, 5 days/week for 1 year (Naylor and Stout 1996).

In a 2-generation study, 1,4-DCB was administered by daily gavage in olive oil to male and female Sprague-Dawley rats at dose levels of 0, 30, 90, or 270 mg/kg/day (Bornatowicz et al. 1994). Groups of 24 F_0 rats/sex/ dose were treated for 77 days (males) and 14 days (females) before mating, followed by exposure of both sexes for 21 days during mating and females during gestation. Groups of 24 F_1 weanlings/sex/dose were treated for 84 days before mating, followed by exposure of both sexes for 30 days during mating and females during gestation (21 days) and lactation (21 days). There were no effects on mating or fertility in either generation as shown by duration between mating and successful

copulation, and fertility index (percentage of pregnant animals out of the number of inseminated animals). Additional reproductive indices were not evaluated as the emphasis of the study was on postnatal developmental toxicity. As discussed in Section 3.2.2.6, developmental effects included reduced birth weight in F_1 pups and increased F_2 pup deaths between birth and postnatal day 4 at ≥90 mg/kg/day.

3.2.2.6 Developmental Effects

1,2-Dichlorobenzene. No studies were located regarding developmental effects in humans after oral exposure to 1,2-DCB.

A limited amount of information is available on the prenatal developmental effects of 1,2-DCB in animals. In a gavage study inadequately reported as an abstract, Sprague-Dawley rats were administered 50, 100, or 200 mg/kg/day of 1,2-DCB on days 6–15 of gestation (Ruddick et al. 1983). Maternal end points included body weight gain, 15 unspecified biochemical parameters, and histology. Fetal toxicity was assessed by evaluating litter size, fetal weight, deciduoma, and skeletal, visceral, and histological changes. The maternal and fetal histological examinations included liver and thyroid; other tissues were not specified. No teratological effects or maternal toxicity were reported. Additional relevant information on the design and results of this study was not included in the abstract.

1,3-Dichlorobenzene. No studies were located regarding developmental effects in humans after oral exposure to 1,3-DCB.

The developmental toxicity study of 1,3-DCB is from a gavage study inadequately reported as an abstract (Ruddick et al. 1983). Sprague-Dawley rats were administered 50, 100, or 200 mg/kg/day of 1,2-DCB on days 6–15 of gestation (use of controls not specified). Maternal end points included body weight gain, 15 unspecified biochemical parameters, and histology. Fetal toxicity was assessed by evaluating litter size, fetal weight, deciduoma, and skeletal, visceral, and histological changes. The maternal and fetal histological examinations included liver and thyroid; other tissues were not specified. No teratological effects or maternal toxicity were reported. Additional relevant information on the design and results of this study was not included in the abstract.

1,4-Dichlorobenzene. No studies were located regarding developmental effects in humans after oral exposure to 1,4-DCB.

A dose-related increase in the incidence of an extra rib was observed in the fetuses of pregnant CD rats administered 1,4-DCB by gavage on Gd 6–15 at doses of 500, 750, and 1,000 mg/kg/day (Giavini et al. 1986). A reduction in fetal weight was observed at 1,000 mg/kg/day. The reduction in fetal weight was not considered to be a fetotoxic effect since it was associated with a decrease in maternal weight gain at the same dosage level. The structural anomaly observed in these fetuses was dose-dependant, but was not considered to be a true adverse effect by the authors. However, these results raise the question of whether 1,4-DCB ingested by the dams reached developing fetal tissue and elicited a developmental effect.

Additional information on prenatal developmental effects of orally administered 1,4-DCB is available from a gavage study inadequately reported as an abstract (Ruddick et al. 1983). Sprague-Dawley rats were administered 50, 100, or 200 mg/kg/day of 1,4-DCB on days 6–15 of gestation (use of controls not specified). Maternal end points included body weight gain, 15 unspecified biochemical parameters, and histology. Fetal toxicity was assessed by evaluating litter size, fetal weight, deciduoma, and skeletal, visceral, and histological changes. The maternal and fetal histological examinations included liver and thyroid; other tissues were not specified. No teratological effects or maternal toxicity were reported. Additional relevant information on the design and results of this study was not included in the abstract.

In a dietary study of 1,4-DCB, female Wistar rats were exposed to a reported estimated dose of 2 mg/kg/day from Gd 1 to Pnd 21 for a total of 42 days (Makita 2005). There were no maternal effects as shown by clinical signs or changes in body weight and food consumption. No fetal examinations were performed but perinatal evaluations showed no gross external malformations or effects on litter size, sex ratio, or pup viability on Pnd 1. Postnatal assessments of the offspring until 6 weeks of age showed no effects on body weight gain, anogenital distance, times of eye and vaginal opening and preputial separation, or serum levels of reproductive hormones (LH and FSH in both sexes and testosterone in males at 6 weeks). Examination of the liver, kidneys, spleen, thymus, testes, epididymides, prostate, seminal vesicles, ovaries, uterus, and thymus at 6 weeks showed no effects on organ weight or histology, except for increased absolute thymus weight (approximately 20% higher than controls) in the female pups. The biological significance of this effect is unclear because it did not occur in the male offspring and was not accompanied by any histological changes.

A 2-generation study was conducted in which 1,4-DCB in olive oil was administered by daily gavage to male and female Sprague-Dawley rats at dose levels of 0, 30, 90, or 270 mg/kg/day (Bornatowicz et al. 1994). Groups of 24 F_0 rats/sex/dose were treated for 77 days (males) and 14 days (females) before mating, followed by exposure of both sexes for 21 days during mating and of females during gestation.

Exposure in the F_0 females was continued throughout lactation until weaning of the F_1 pups on postnatal day 21. Groups of 24 F_1 weanlings/sex/dose were treated for 84 days before mating, followed by exposure of both sexes for 30 days during mating, and of females during gestation and lactation. The study was ended following weaning of the F_2 pups on postnatal day 21. The F_0 and F_1 males were sacrificed 21 days after the end of the mating period (it is unclear if exposure continued postmating), and the F_0 and F_1 females were sacrificed after their pups were weaned. Study end points included clinical observations in adults and pups, body weight and food consumption in maternal animals (during gestation and lactation) and pups (from birth to day 21), reproductive indices, gestation length, litter size, numbers of live and dead pups, postnatal survival, postnatal developmental milestones (times to erect ears and eyelid separation), and neurobehavioral effects in pups at weaning (auricle reflex, orientation reaction, grasping, and draw-up reflexes). Necropsies were performed on all adult males and females, as well as on pups that died during the first 4 days or were killed on day 4 (i.e., those not selected for continuation in the study). Liver, kidney, and spleen weights were measured in males and females of both generations. Histopathological examinations were performed on selected tissues (liver, kidneys, spleen, vagina, cervix, uterus, ovaries, mammary gland, testes, epididymides, penis, prostate, seminal vesicles, and spermatic cord) from F_0 and F_1 adult animals that had no living young, died prematurely, or were killed as moribund, as well as on gross lesions in all animals.

There were no exposure-related effects in adult rats or pups at 30 mg/kg/day (Bornatowicz et al. 1994). Body weight was significantly reduced in F_1 pups at birth at ≥90 mg/kg/day (4.4, 5.7, and 22.6% lower than control group at 30, 90, and 270 mg/kg/day), in F_1 pups on postnatal days 7–21 at 270 mg/kg/day, and in F_2 pups at birth and on postnatal days 4–21. The total number of deaths from birth to postnatal day 4 was significantly increased in F_1 pups at 270 mg/kg/day and F_2 pups at ≥90 mg/kg/day (33, 467, and 1,033% higher than controls at 30, 90, and 270 mg/kg/day). None of the data in this study were reported on a per-litter basis or analyzed for dose-related trends. Decreased offspring survival at 270 mg/kg/day is also indicated by reduced total number of live F_1 and F_2 pups at birth, increased total dead F_1 and F_2 pups at birth, and increased total dead F_1 and F_2 pups during postnatal days 5–21. Other postnatal effects in the offspring included delayed eye opening (first day of appearance or day shown in all pups) in F_1 and F_2 pups at 270 mg/kg/day, delayed ear erection (day shown in all pups) in F_2 pups at 270 mg/kg/day, and reduced percentage of rats per litter with a positive draw-up reflex in the F_1 pups at 270 mg/kg/day and in F_2 pups at ≥90 mg/kg/day. Clinical manifestations occurred in pups of both generations at ≥90 mg/kg/day, including dry and scaly skin until approximately postnatal day 7 (0, 0, ≈70, and 100% of the litters at 0, 30, 90, and 270 mg/kg/day), and tail constriction that appeared between days 4 and 21 in all or nearly all litters (percentages not reported) and occasionally led to loss of parts of the tail.

Additionally, the number of F_1 pups described as cyanotic after birth was increased (not quantified) at 270 mg/kg/day.

Effects in adult animals were generally not quantified, but included reduced average body weight in F_1 males and females at 270 mg/kg/day at all time points during gestation and lactation, increased relative liver weight in F_1 males at ≥90 mg/kg/day, and changes in absolute and/or relative organ weights in kidneys (increased) and spleen (reduced) in F_1 males at 270 mg/kg/day. There were no effects on organ weights in female rats of either generation. The only histopathological finding attributed to exposure was unspecified kidney damage in both generations (effect levels, possible male specificity, and other information not reported). There were no effects on mating and fertility indices in any group (see Section 3.2.2.5).

3.2.2.7 Cancer

1,2-Dichlorobenzene. No studies were located regarding carcinogenic effects in humans after oral exposure to 1,2-DCB.

Carcinogenicity was evaluated in groups of 50 male and 50 female F344/N rats and 50 male and 50 female $B6C3F_1$ mice that were exposed to 1,2-DCB (>99% pure) in corn oil by gavage in doses of 0, 60, or 120 mg/kg, 5 days/week for 103 weeks (NTP 1985). Evaluations in both species included clinical signs, body weight, and necropsy and histology on all animals. As discussed below, no exposure-related tumors were found in either species, although it is unclear whether a maximum tolerated dose (MTD) was achieved in either species.

In rats, survival to termination in the high-dose males was significantly reduced compared with controls (19/50 vs. 42/50, $p<0.001$), but NTP (1985) concluded that the difference was likely mainly from causes incidental to treatment. Due to the probable gavage-related deaths in the high-dose male rats, the lower survival of this group does not necessarily mean that the MTD was either reached or exceeded. No clinical signs were reported. Mean body weight was slightly reduced (≈5% less than controls) in males throughout the study at 85.7 mg/kg/day; the only effect in females was a small increase compared to controls after week 32 in both dose groups (final body weights were 11–12% increased at 42.9 and 85.7 mg/kg/day). There were no exposure-related increased tumor incidences in the rats. The incidence of adrenal gland pheochromocytomas was significantly ($p\leq0.05$) increased in low-dose males by the life table test (mortality adjusted incidence of 20.9, 40.5, and 21.7% in the control, low-dose, and high-dose

groups, respectively), but not statistically significant by the incidental tumor test, which was considered to be the more appropriate mortality-adjusted test for analysis of nonfatal types of tumors. The increased incidence of pheochromocytomas in the low-dose males also was not significant in the Fisher Exact test (without mortality adjustment), and there was no significant dose-related trend in the Cochran-Armitage test. No increase in pheochromocytomas was seen in high-dose males. The increased incidence of pheochromocytomas in the low-dose male rats was discounted by NTP (1985) because there was no dose-response trend or high-dose effect, no increased incidence in females, no observation of malignant pheochromocytomas, and questionable toxicological significance of the life table test results (pheochromocytomas were not considered to be a life-threatening condition). Incidences of interstitial-cell tumors of the testis were elevated in control and treated groups (47/50, 49/50, 41/50), and occurred with a significant positive trend when analyzed by the life-table test. However, the increase detected by the life-table test was discounted by NTP because this tumor is not considered to be life threatening, and no significant results were obtained by the incidental tumor test, which is the more appropriate test for nonfatal tumors. The Cochran-Armitage test showed a significant negative trend for the interstitial cell tumors.

There were no clinical signs or effects on body weight or survival in the mice, indicating that it is unclear whether an MTD was achieved in this species (NTP 1985). There were no clear compound-related increased incidences of neoplasms in the mice. Incidences of malignant histiocytic lymphomas showed a significant positive dose-related trend in male mice (0/50, 1/50, 4/50) and female mice (0/49, 0/50, 3/49), but NTP considered numbers of animals with all types of lymphomas to be a more appropriate basis for comparison. Because malignant lymphocytic lymphomas occurred in male mice (7/50, 0/50, 0/50) with a significant negative dose-related trend, and the combined incidence of all types of lymphomas was not significantly different than that in controls for the male mice (8/50, 2/50, 4/50) or female mice (11/49, 11/50, 13/49) by any of the statistical tests, the increase in histiocytic lymphomas was discounted by NTP. Alveolar/bronchiolar carcinomas were significantly increased in the high dose male mice (4/50, 2/50, 10/50). The incidences showed a significant positive increasing trend by the Cochran-Armitage test, but not by the life-table or incidental tumor test. The increase in alveolar/bronchiolar carcinomas was discounted by NTP because the more appropriate combined incidence of male mice with alveolar/bronchiolar adenomas or carcinomas (8/50, 8/50, 13/50) was not significantly greater than controls in any of the tests.

1,3-Dichlorobenzene. No studies were located regarding carcinogenic effects in humans or animals after oral exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding carcinogenic effects in humans after oral exposure to 1,4-DCB.

1,4-DCB was found to be carcinogenic in $B6C3F_1$ mice and male (but not female) F344 rats exposed to 1,4-DCB for 2 years in a carcinogenesis bioassay (NTP 1987). 1,4-DCB was administered by gavage to male rats at doses of 150 or 300 mg/kg/day and female rats at doses of 300 or 600 mg/kg/day. Significant dose-related increases in the incidence of renal tubular cell adenocarcinomas were reported in male rats (controls, 2%; low-dose, 6%; high-dose, 14%). Spontaneous tumors of this type are uncommon in male F344 rats; they have been diagnosed in only 4 of 1,098 (0.4%) of the corn oil-gavage controls in previous NTP studies. There were no tubular cell tumors in dosed or vehicle-control female rats. There also was a marginal increase in the incidence of mononuclear cell leukemia in dosed male rats that was only slightly higher than the incidence in historical controls from the same laboratory. The NTP study concluded that 1,4-DCB was carcinogenic in male rats, but not in female rats.

In a 2-year bioassay in $B6C3F_1$ mice that received 1,4-DCB at 300 or 600 mg/kg/day (NTP 1987), increased incidences of hepatocellular carcinomas were observed in high-dose male mice (controls, 28%; low-dose, 22.5%; high-dose, 64%) and high-dose female mice (controls, 10%; low-dose, 10.4%; high-dose, 38%). Hepatocellular adenomas were increased in high- and low-dose male mice (controls, 10%; low-dose, 26.2%; high-dose, 32%) and in high-dose female mice (controls, 20%; low-dose, 12.5%; high-dose, 42%). Female control mice in this bioassay had a substantially higher incidence of liver tumors than did historical controls. Hepatoblastomas (a rare form of hepatocellular carcinoma) were observed in four high-dose male mice along with other hepatocellular carcinomas. This tumor type had not been previously observed in 1,091 male vehicle-control mice in NTP studies. An increase in thyroid gland follicular cell hyperplasia was observed in dosed male mice, and there was a marginal positive trend in the incidence of follicular cell adenomas of the thyroid gland in female mice. The incidence of pheochromocytomas (tumors of chromaffin tissue of the adrenal medulla or sympathetic prepangliar, benign and malignant, combined) of the adrenal gland was 0 of 47 (control), 2 of 48 (low dose), and 3 of 49 (high dose), and the incidence of adrenal gland medullary hyperplasia and focal hyperplasia of the adrenal gland capsule were increased as well in dosed male mice.

The observation that kidney tumors are induced in male rats, but not female rats, in response to exposure to certain chemicals has been the subject of recent research. It has been hypothesized that the male rat kidney is susceptible to the induction of certain tumors because it contains the protein $\alpha_{2\mu}$-globulin, which

has not been found at significant levels in either female rats, or in mice and humans of either sex (Charbonneau et al. 1987, 1989a, 1989b). Chemicals like 1,4-DCB, which reversibly bind to this protein, cause the formation of hyalin droplets in the proximal convoluted tubules of male rats. The hyalin droplet-protein complex is resistant to degradation by lysosomal enzymes and accumulates in the tubule, leading to localized hyperplasia of the epithelium (Borghoff et al. 1991; EPA 1991i). It is hypothesized that the resulting cellular damage and cell proliferation enhances tumor formation via a mechanism not yet elucidated. It has also been demonstrated that the same effects can be elicited in male rats administered other $\alpha_{2\mu}$-globulin-binding chemicals such as [hexachloroethane, d-limonene 1-methyl-4(1-methylethenyl)cyclohexene], unleaded gasoline, and pentachloroethane (EPA 1991i). Based on these data, EPA (1991) concluded that tumors associated with $\alpha_{2\mu}$-globulin and hyalin droplets are specific to species that produce this protein in large quantities, and that these tumors should be distinguished from other renal tumors.

The finding of hepatocellular carcinomas and adenomas in mice in the NTP (1987) study has been the subject of scientific debate. There was a high incidence of these tumors in both male and female control animals, but this is fairly common in mice. However, in this case, the tumor incidence in the female controls was substantially higher than the historical control value. In addition, 1,4-DCB has not been demonstrated to be mutagenic in any of the microbial or mammalian systems tested (NTP 1987), suggesting that the liver tumors are not the result of genotoxicity. Hepatocellular degeneration with resultant initiation of tissue repair was present in both male and female treated mice. This led NTP (1987) to speculate that 1,4-DCB acted as a tumor promotor rather than a tumor initiator during the formation of the liver tumors found in male and female mice.

As shown in Table 3-5, 300 mg/kg/day is the cancer effect level (CEL) for renal tubular cell adenomas in male rats and 600 mg/kg/day is the CEL for hepatocellular carcinomas and hepatoblastomas in mice (NTP 1987).

3.2.3 Dermal Exposure

3.2.3.1 Death

1,2-Dichlorobenzene. No studies were located regarding death in humans or animals after dermal exposure to 1,2-DCB.

1,3-Dichlorobenzene. No studies were located regarding death in humans or animals after dermal exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding death in humans after dermal exposure to 1,4-DCB.

The dermal LD_{50} for 1,4-DCB in Sherman rats was >6,000 mg/kg/day (Gaines and Linder 1986). It is not clear how many rats died after dermal exposure to 1,4-DCB in this study, and there are no toxicokinetic data that address the question of absorption of 1,4-DCB by the dermal route.

3.2.3.2 Systemic Effects

1,2-Dichlorobenzene. No studies were located regarding systemic toxicity in humans or animals after dermal exposure to 1,2-DCB.

Application of two drops of undiluted 1,2-DCB into the eyes of rabbits caused some pain and slight irritation of the conjunctival membranes, which healed completely within 1 week (Hollingsworth et al. 1958). The irritation was reduced by prompt rinsing with water. Additional relevant information was not reported.

1,3-Dichlorobenzene. No studies were located regarding systemic effects in humans or animals after dermal exposure to 1,3-DCB.

1,4-Dichlorobenzene. No studies were located regarding systemic effects in humans or animals after dermal exposure to 1,4-DCB.

Industrial experience indicates that solid particles of 1,4-DCB are painful in the eyes of humans (Hollingsworth et al. 1956). Solid 1,2-DCB has a negligible irritating action on intact, uncovered human skin, but can produce a burning sensation when held in close dermal contact for an unspecified excessive period of time (Hollingsworth et al. 1956). Prolonged and repeated contact to strong solutions of 1,4-DCB also could cause slight irritation in intact skin (Hollingsworth et al. 1956).

No studies were located regarding the following health effects in humans or animals after dermal exposure to 1,2-, 1,3-, or 1,4-DCB:

3.2.3.3 Immunological and Lymphoreticular Effects

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.3 GENOTOXICITY

In vivo and *in vitro* genotoxicity studies of DCBs are summarized in Tables 3-6 and 3-7, respectively.

1,2-Dichlorobenzene. No studies were located regarding genotoxic effects in humans after inhalation, oral, or dermal exposure to 1,2-DCB.

A limited amount of information is available on the genotoxicity of 1,2-DCB in animals. Micronuclei were induced in bone marrow erythrocytes of mice that were administered two 93.5–375 mg/kg doses by intraperitoneal injection 24 hours apart; lower dose levels were not tested (Mohtashamipur et al. 1987). A single 0.4 mg/kg intraperitoneal dose of 1,2-DCB caused covalent binding to liver, lung, kidney, and stomach DNA in rats and mice (Colacci et al. 1990).

In vitro reverse mutation assays of 1,2-DCB in microbial systems were negative in *Salmonella typhimurium* with or without metabolic activation (Connor et al. 1985; NTP 1985; Shimizu et al. 1983; Waters et al. 1982), negative in *Escherichia coli* without metabolic activation (Waters et al. 1982), and positive results in *Saccharomyces cerevisiae* with metabolic activation (Paolini et al. 1998). In mouse lymphoma cells, 1,2-DCB was negative for forward mutation without metabolic activation, but positive with S9 activation mixture (Myhr and Caspary 1991). *In vitro* exposure to 1,2-DCB induced DNA damage in *E. coli* and *S. cerevisiae*, but not in *Bacillus subtilis* (Waters et al. 1982), and did not cause replicative DNA synthesis in cultured human lymphocytes (Perocco et al. 1983) or increased DNA repair in primary rat hepatocytes (Williams et al. 1989). 1,2-DCB did not cause chromosomal aberrations, either with or without metabolic activation, in Chinese hamster ovary (CHO) cells, but did induce sister-chromatid exchanges only in the presence of S9 metabolic activation preparation (Loveday et al. 1990).

Table 3-6. Genotoxicity of Dichlorobenzenes In Vivo

Species (test system)	End point	Results	Reference
1,2-Dichlorobenzene			
Mammalian cells			
Mouse bone marrow erythrocytes[a]	Micronucleus formation	+	Mohtashampir et al. 1987
Rat liver, lung, kidney and stomach cells[b]	Covalent binding to DNA	+	Colacci et al. 1990
Mouse liver, lung, kidney and stomach cells[b]	Covalent binding to DNA	+	Colacci et al. 1990
1,3-Dichlorobenzene			
Mammalian cells			
Mouse bone marrow erythrocytes[c]	Micronucleus formation	+	Mohtashampir et al. 1987
1,4-Dichlorobenzene			
Mammalian cells			
Rat bone marrow cells[d]	Chromosomal aberrations	–	Anderson and Richardson 1976
Mouse bone marrow cells	Micronucleus formation	–	Shelby and Witt 1995
Mouse erythrocytes[e]	Micronucleus formation	–	NTP 1987
Rat kidney cells[f]	Unscheduled DNA synthesis	–	Steinmetz and Spanggord 1987b
	Increased DNA replication	+[g]	
Mouse hepatocytes[h]	Unscheduled DNA synthesis	–	Steinmetz and Spanggord 1987a
Rat kidney cells[i]	Increased DNA replication	+	Charbonneau et al. 1989b
Mouse bone marrow erythrocytes[j]	Micronucleus formation	+	Mohtashamipur et al. 1987
Rat renal tubular cells and hepatocytes[k]	Cumulative replicating fraction	–	Umemura et al. 1998
Mouse renal tubular cells and hepatocytes[k]	Cumulative replicating fraction	+	Umemura et al. 1998

[a]Exposed to 1,2-dichlorobenzene via two intraperitoneal injections of 93.5, 187.5, 281, or 375 mg/kg (24 hours apart) and sacrificed 6 hours after the second injection. Males only were tested.
[b]Exposed to 1,2-dichlorobenzene via one intraperitoneal injection of 0.4 mg/kg.
[c]Exposed to 1,3-dichlorobenzene via two intraperitoneal injections of 87.5, 175, 262.5, or 700 mg/kg (24 hours apart) and sacrificed 6 hours after the second injection. Males only were tested.
[d]Exposed to 1,4-dichlorobenzene via inhalation for 2 hours at 299 or 682 ppm; for 5 days, 5 hours/day at 75 or 500 ppm; or for 3 months, 5 days/week, 5 hours/day at 75 or 500 ppm.
[e]Exposed to 1,4-dichlorobenzene via gavage for 13 weeks, 5 days/week at 600–1,800 mg/kg/day.
[f]Exposed to 1,4-dichlorobenzene via gavage in corn oil at 300, 600, or 1,000 mg/kg at 16 hours before sacrifice for unscheduled DNA synthesis experiment or at 96 hours before sacrifice for DNA replication experiment.
[g]Results were positive for male rats only in which a significant S-phase response was induced.
[h]Exposed to 1,4-dichlorobenzene via gavage in corn oil at 300, 600, or 1,000 mg/kg at 16 or 48 hours before sacrifice.
[i]Exposed to 1,4-dichlorobenzene via gavage in corn oil at 120 or 300 mg/kg/day for 7 days and sacrificed 24 hours after the last dose.
[j]Exposed to 1,4-dichlorobenzene via two intraperitoneal injections of 355, 710, 1,065, or 1,420 mg/kg (24 hours apart) and sacrificed 6 hours after the second injection. Males only were tested.
[k]Exposed to 1,4-dichlorobenzene via gavage for 1 week or 4 weeks at 150, 300, or 600 mg/kg/day.

+ = positive result; – = negative result; DNA = deoxyribonucleic acid

Table 3-7. Genotoxicity of Dichlorobenzenes In Vitro

Species (test system)	End point	Results		Reference
		With activation	Without activation	
1,2-Dichlorobenzene				
Microbial systems				
Salmonella typhimurium TA98, TA100, TA1535, TA1537, and TA1538	Gene mutation	ND	–	Waters et al. 1982
S. typhimurium TA98, TA100, UTH8413, and UTH8414	Gene mutation	–	–	Connor et al. 1985
S. typhimurium TA98, TA100, TA1535, and TA1537	Gene mutation	–	–	NTP 1985
S. typhimurium TA98, TA100, TA1535, TA1537, and TA1538	Gene mutation	–	–	Shimizu et al. 1983
S. typhimurium	Gene induction (*umu*)	–	–	Nakamura et al. 1987
Escherichia coli	Prophage lambda induction			DeMarini and Brooks 1992
E. coli WP2 *uvra*	Gene mutation	ND	–	Waters et al. 1982
*E. coli pol*A$^-$	DNA damage	ND	+	Waters et al. 1982
*Bacillus subtilis rec*A$^-$	DNA damage	ND	+	Waters et al. 1982
Saccharomyces cerevisiae	Gene mutation	–	ND	Paolini et al. 1998
S. cerevisiae D3	DNA damage	ND	+	Waters et al. 1982
Mammalian cells				
Mouse lymphoma cells	Gene mutation	+	–	Myhr and Caspary 1991
Chinese hamster ovary cells	Chromosomal aberrations	–	–	Loveday et al. 1990
Chinese hamster ovary cells	Sister-chromatid exchange	+	–	Loveday et al. 1990
Rat primary hepatocytes	Increased DNA repair	ND	–	Williams et al. 1989
Human lymphocytes	Replicative DNA synthesis	–	–	Perocco et al. 1983
1,3-Dichlorobenzene				
Microbial systems				
S. typhimurium TA98, TA100, TA1535, TA1537, and TA1538	Gene mutation	ND	–	Waters et al. 1982
S. typhimurium TA98, TA100, UTH8413, and UTH8414	Gene mutation	–	–	Connor et al. 1985

Table 3-7. Genotoxicity of Dichlorobenzenes In Vitro

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
S. typhimurium TA98, TA100, TA1535, TA1537, and TA1538	Gene mutation	–	–	Shimizu et al. 1983
E. coli WP2 *uvra*	Gene mutation	ND	–	Waters et al. 1982
*E. coli pol*A$^-$	DNA damage	ND	+	Waters et al. 1982
*B. subtilis rec*A$^-$	DNA damage	ND	+	Waters et al. 1982
S. cerevisiae D3	DNA damage	ND	–	Waters et al. 1982
Mammalian cells				
Human lymphocytes	Replicative DNA synthesis	–	–	Perocco et al. 1983
1,4-Dichlorobenzene				
Microbial systems				
S. typhimurium[a] TA98, TA100, TA1535, and TA1538	Gene mutation	–	–	Anderson 1976
S. typhimurium[b] TA98, TA100, and TA1538	Gene mutation	–	–	Anderson 1976
S. typhimurium[b] TA1535	Gene mutation	+	–	Anderson 1976
S. typhimurium TA98, TA100, TA1535, TA1537, and TA1538	Gene mutation	–	–	Shimizu et al. 1983; Waters et al. 1982
S. typhimurium TA98, TA100, TA1535, and TA1537	Gene mutation	–	–	Haworth et al. 1983; NTP 1987
S. typhimurium TA98, TA100, UTH8413, and UTH8414	Gene mutation	–	–	Connor et al. 1985
E. coli WP2 *uvra*	Gene mutation	ND	–	Waters et al. 1982
*E. coli pol*A$^-$	DNA damage	ND	-	Waters et al. 1982
*B. subtilis rec*A$^-$	DNA damage	ND	-	Waters et al. 1982
S. cerevisiae	Gene mutation	+	ND	Paolini et al. 1998
S. cerevisiae D3	DNA damage	ND	-	Waters et al. 1982
Mammalian cells				
mouse lymphoma cells L5178Y/TK$^\pm$	Gene mutation	(=)	–	NTP 1987
mouse lymphoma cells L5178Y/TK$^\pm$	Gene mutation	+	(=)	McGregor et al. 1988
Chinese hamster lung cells	Gene mutation	–	–	Instituto di Ricerche Biomediche 1986b
Chinese hamster ovary cells	Chromosomal aberrations	–	–	Anderson et al. 1990; NTP 1987

Table 3-7. Genotoxicity of Dichlorobenzenes In Vitro

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Chinese hamster ovary cells	Sister chromatid exchanges	–	–	Anderson et al. 1990; NTP 1987
Rat hepatocytes	DNA fragmentation	ND	-	Canonero et al. 1997
Rat hepatocytes	Micronucleus formation	ND	(=)	Canonero et al. 1997
Rat kidney cells	DNA damage	ND	+	Robbiano et al. 1997
Rat kidney cells	Micronucleus formation	ND	+	Robbiano et al. 1997
Human kidney cells	DNA damage	ND	+	Robbiano et al. 1997
Human kidney cells	Micronucleus formation	ND	+	Robbiano et al. 1997
Human hepatocytes	DNA fragmentation	ND	-	Canonero et al. 1997
Human hepatocytes	Micronucleus formation	ND	-	Canonero et al. 1997
Human lymphocytes	Replicative DNA synthesis	–	–	Perocco et al. 1983
Human lymphocytes	Sister-chromatid exchanges	–	–	Carbonell et al. 1991
Human lymphocytes	Unscheduled DNA synthesis	–	–	Perocco et al. 1983; Instituto di Ricerche Biomediche 1987
HeLa cells	Unscheduled DNA synthesis	–	–	Instituto di Ricerche Biomediche 1986a
Plant systems				
Root tips (16 species of dicotyledons and monocotyledons)	Chromosomal aberrations	ND	+	Sharma and Battachary 1956
Lens esculenta (L.) Moench	Mitotic abnormalities	ND	+	Sarbhoy 1980
Aspergillus nidulans	Back mutation frequency	ND	+	Prasad 1970
Tribe viceae	Chromosomal aberrations	ND	+	Srivastava 1966

[a]Exposed to 1,4-dichlorobenzene gas.
[b]Exposed to 1,4-dichlorobenzene in DMSO.

– = negative result; + = positive result; (=) = equivocal; DNA = deoxyribonucleic acid; ND = not determined

1,3-Dichlorobenzene. No studies were located regarding genotoxic effects in humans after inhalation, oral, or dermal exposure to 1,3-DCB.

A limited amount of information is available on the genotoxicity of 1,3-DCB. Micronuclei were induced in bone marrow erythrocytes of mice following administration of two 87.5–700 mg/kg doses by intraperitoneal injection 24 hours apart; lower dose levels were not tested (Mohtashamipur et al. 1987). *In vitro* exposure to 1,3-DCB did not induce reverse mutations in *S. typhimurium* (Connor et al. 1985; Shimizu et al. 1983; Waters et al. 1982) or *E. coli* (Waters et al. 1982). 1,3-DCB caused DNA damage in *E. coli*, but not in *B. subtilis* or *S. cerevisiae* (Waters et al. 1982), and did not increase replicative DNA synthesis in cultured human lymphocytes (Perocco et al. 1983).

1,4-Dichlorobenzene. No studies were located regarding genotoxic effects in humans after inhalation, oral, or dermal exposure to 1,4-DCB.

Cytogenetic studies have been conducted using bone marrow cells of rats following inhalation exposure to 1,4-DCB (Anderson and Richardson 1976). Three series of exposures were carried out: (1) one exposure at 299 or 682 ppm for 2 hours; (2) exposures at 75 or 500 ppm, 5 hours/day for 5 days; and (3) exposures to 75 or 500 ppm, 5 hours/day, 5 days/week for 3 months. Bone marrow cells from both femurs were examined for chromosome or chromatid gaps, chromatid breaks, fragments, or other complex abnormalities. In all three experiments, exposure to 1,4-DCB failed to induce any effects indicative of chromosomal damage.

Gavage administration of 1,4-DCB to B6C3F_1 mice and F344 rats at single doses of 300–1,000 mg/kg/day did not result in unscheduled deoxyribonucleic acid (DNA) synthesis in the mouse hepatocytes or in the renal tissue of the rats in an *in vivo*/*in vitro* assay (Steinmetz and Spanggord 1987a, 1987b). However, 1,4-DCB at the highest level did induce an increase in DNA replication (S-phase of cell division) in the renal tissue of the male rats and in the hepatocytes of the male mice. Based on a comparison with historical controls, the authors concluded that levels of DNA replication were also significantly elevated in the hepatocytes of female mice.

No evidence of a clastogenic effect was found in mouse bone marrow erythroblasts after a single gavage administration of 1,4-DCB at 2,500 mg/kg/day (Herbold 1986a). Similarly, no evidence of clastogenic effects was found in mouse bone erythroblasts after a single oral administration of 2,5-dichlorophenol

(the major metabolite of 1,4-DCB) at 1,500 mg/kg/day (Herbold 1986b). 2,5-Dichlorophenol with or without metabolic activation did not induce an increase in mutagenic response in the Chinese hamster ovary HGPRT forward mutation assay (Litton Bionetics 1986a). This compound was also inactive in the Balb/3T3 *in vitro* transformation assay (Litton Bionetics 1985).

Cytogenetic effects were not found in bone marrow cells from mice treated with 1,4-DCB by gavage at levels up to 1,800 mg/kg/day in a 13-week study (NTP 1987). No increase in micronucleated cells occurred even at levels that were extremely toxic to the test animals, resulting in liver toxicity and decreased survival rates. As noted by the authors of that study, the observed carcinogenic activity of 1,4-DCB cannot be adequately predicted on the basis of the available genotoxicity data; all of the available information strongly suggests that 1,4-DCB acts as a tumor promoter rather than as a mutagen.

However, gavage administration of a single 1,000 mg/kg/day dose of 1,4-DCB to mice and rats resulted in an increase in DNA replication in the renal tissue of the male rats and in the hepatocytes of mice of both sexes (Steinmetz and Spanggord 1987a, 1987b). Increased ^{3}H-thymidine incorporation into renal DNA has also been demonstrated in rats dosed with 1,4-DCB by gavage at 120 mg/kg/day for 7 days (Charbonneau et al. 1989b). These observations suggest that 1,4-DCB promotes cell division, a finding that may help to elucidate the mechanism of carcinogenic action of 1,4-DCB in male rat kidneys and mouse liver in the NTP (1987) bioassay. However, it is important to note that in these studies; only kidney tissue was tested in the rat for increased DNA replication, and in the mouse, only liver tissue was tested. Therefore, it is not clear whether increased cell replication also occurs in other tissue in each species or is limited to the tissues in which the carcinogenic effects occurred.

The *in vivo* genotoxicity of 1,4-DCB is summarized in Table 3-6. As discussed above, the *in vivo* testing showed positive results for increased DNA replication in the livers of orally exposed mice (Steinmetz and Spanggord 1987a) and in the kidneys of orally exposed rats (Charbonneau et al. 1989b; Steinmetz and Spanggord 1987b), and mixed positive and negative findings for induction of micronuclei in bone marrow cells of orally exposed mice (Mohtashampir et al. 1987; NTP 1987).

In vitro genotoxicity studies of 1,4-DCB are summarized in Table 3-7. Microbial reverse mutation tests were predominantly negative in *S. typhimurium* (Anderson 1976; Connor et al. 1985; NTP 1987; Shimizu et al. 1983; Waters et al. 1982) and *E. coli* (Waters et al. 1982), but positive in *S. cerevisiae* (Paolini et al. 1998). Assays for DNA damage in *E. coli*, *B. subtilis*, and *S. cerevisiae* were negative (Waters et al. 1982). 1,4-DCB did not induce replicative DNA synthesis (Perocco et al. 1983) or DNA strand breaks

(Canonero et al. 1997) in rat and human hepatocytes, although DNA damage was increased in rat and human kidney cells (Robbiano et al. 1999). Forward mutation assays in mouse lymphoma cells were equivocal (McGregor et al. 1988; NTP 1987), and mixed positive and negative results were found for chromosomal aberrations and sister-chromatid exchanges in CHO cells (Anderson et al. 1990; Carbonell et al. 1991; NTP 1987). Tests for micronucleus formation were equivocal in human and rat hepatocytes (Canonero et al. 1997) and positive in human and rat kidney cells (Robbiano et al. 1999). *In vitro* testing in plant systems showed genotoxic effects that included chromosomal aberrations, mitotic abnormalities, and back mutations (Prasad 1970; Sarbhoy 1980; Sharma and Battacharya 1956; Srivastava 1966).

3.4 TOXICOKINETICS

1,2-DCB is quickly and extensively absorbed through both the gastrointestinal tract and the respiratory tract; studies describing the absorption of 1,2-DCB following dermal exposure are not available. Following absorption, 1,2-DCB is distributed throughout the body, but tends to be found in greatest levels in the fat, kidney, and liver. 1,2-DCB is initially metabolized by cytochrome P-450 enzymes, specifically P4502E1, to an active epoxide followed by hydrolysis to 2,3-dichlorophenol or 3,4-dichlorophenol. The dichlorophenols may be further oxidized or, more often, be conjugated to glutathione, sulfate, or to form the glucuronide; conjugation occurs extensively, with virtually no unconjugated metabolites reported in the available studies. Metabolism is believed to occur mainly in the liver, but may occur at lower levels in other tissues, such as the kidney or lung. Elimination of 1,2-DCB from the body is rapid, with the majority of a single dose being removed within the first 75 hours postexposure; elimination occurs primarily in the urine as metabolites.

Information on the quantitative absorption of 1,3-DCB in humans and animals is not available for any route of exposure; however, absorption of the compound can be inferred from studies that have detected 1,3-DCB or metabolites in the breast milk, blood, and fat of humans and in the bile and urine of exposed animals. Distribution is believed to be similar to the other DCB isomers, but data demonstrating this are not presently available. Similar to the other DCB isomers, 1,3-DCB is initially metabolized by cytochrome P-450 enzymes, followed by extensive conjugation, primarily to glutathione, has been reported. 1,3-DCB is eliminated mainly in the urine, similar to the other DCB isomers.

Absorption of 1,4-DCB is rapid and essentially complete following inhalation or oral exposure. Information on the quantitative absorption of 1,4-DCB following dermal exposure are not available; however, absorption is believed to be very low, based on a very high (>6 g/kg) dermal LD_{50} for 1,4-DCB

in rats, and on a lack of systemic effects in humans who held solid 1,4-DCB in their hands. Similar to the other dichlorobenzene isomers, 1,4-DCB is distributed throughout the body, but tends to be found in greatest levels in fat, liver, and kidney. Metabolism of 1,4-DCB is similar to that of 1,2-DCB, with an initial oxidation to an epoxide, followed by hydrolysis to 2,5-dichlorophenol. Extensive phase II metabolism occurs subsequently, with eliminated metabolites found mainly as the sulfate, glucuronide, or mercapturic acid. 1,4-DCB is eliminated almost exclusively in the urine, primarily as conjugates of 2,5-dichlorophenol.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

1,2-Dichlorobenzene. Quantitative data on the absorption of 1,2-DCB in humans following inhalation exposure are not available. However, evidence for absorption of 1,2-DCB in humans comes from numerous studies that have detected 1,2-DCB in human tissues, including the blood (Bristol et al. 1982), urine (Kumagai and Matsunaga 1995, 1997; Zenser et al. 1997), adipose tissue (Jan 1983), and in breast milk (Jan 1983; Mes et al. 1986). While these studies do not provide a quantitative measure of the rate or extent of 1,2-DCB and cannot provide information concerning possible exposure route, they provide evidence of 1,2-DCB absorption in humans.

Quantitative data on the absorption of 1,2-DCB in animals are similarly not available. However, numerous studies presenting evidence of systemic toxicity (see Section 3.2) following inhalation of 1,2-DCB provide qualitative evidence for the absorption of 1,2-DCB.

1,3-Dichlorobenzene. Quantitative data on the absorption of 1,3-DCB in humans following inhalation exposure are not available. However, evidence for absorption of 1,3-DCB in humans comes from studies that have detected 1,3-DCB in breast milk (Mes et al. 1986), blood (Bristol et al. 1982), and adipose tissue (Jan 1983). While these studies do not provide a quantitative measure of the rate or extent of 1,3-DCB and cannot provide information concerning possible exposure route, they provide evidence of 1,3-DCB absorption in humans.

Quantitative inhalation absorption data for 1,3-DCB are not available, but absorption characteristics are likely to be similar to those of the other isomers based on similarities in chemical and physical properties.

1,4-Dichlorobenzene. Quantitative data on the absorption of 1,4-DCB in humans following inhalation exposure are not available. However, evidence for absorption of 1,4-DCB in humans comes from numerous studies that have detected 1,4-DCB in human tissues, including the blood (Bristol et al. 1982; Hill et al. 1995), urine (Ghittori et al. 1985; Hill et al. 1995; Pagnotto and Walkley 1965), adipose tissue (Jan 1983), and breast milk (Jan 1983). While these studies do not provide a quantitative measure of the rate or extent of 1,4-DCB and cannot provide information concerning possible exposure route, they provide evidence that 1,4-DCB is absorbed by humans.

Studies presenting quantitative data on the rate and/or extent of absorption of 1,4-DCB following inhalation exposure in animals are not available. However, numerous studies presenting evidence of systemic toxicity (see Section 3.2) following inhalation exposure provide qualitative evidence for the absorption of 1,4-DCB. Additional evidence comes from studies that have reported the presence of the compound or its metabolites in peripheral tissues following inhalation exposure. Following a single or multiple 3-hour inhalation exposures of radiolabeled 1,4-DCB in rats, label was detected in all evaluated tissues (liver, kidneys, lungs, muscle, fat, and blood plasma), indicating that considerable absorption had occurred (Hawkins et al. 1980). Levels of label in tissues did not appreciably increase with increasing the number of exposures beyond one (Hawkins et al. 1980). Similarly, following a single 24-hour inhalation exposure in rats, 1,4-DCB levels in the liver, kidney, fat, and blood increased sharply during the first 6-hour evaluation period, then rose slowly but steadily for the remainder of the exposure period (Umemura et al. 1998), indicating an initial rapid absorption, followed by a slower total absorption as equilibration of body and blood levels is approached.

3.4.1.2 Oral Exposure

1,2-Dichlorobenzene. Quantitative data on the absorption of 1,2-DCB in humans following oral exposure are not available. However, absorption of 1,2-DCB in humans can be concluded based on the results of numerous studies that have detected 1,2-DCB in human tissues, including the blood (Bristol et al. 1982), urine (Kumagai and Matsunaga 1995, 1997; Zenser et al. 1997), and in breast milk (Jan 1983; Mes et al. 1986). While these studies do not provide a quantitative measure of the rate or extent of 1,2-DCB and cannot provide information concerning possible exposure route, they provide evidence of 1,2-DCB absorption in humans.

In male Wistar rats given single oral doses of 5, 50, and 250 mg/kg body weight of 14C-labeled 1,2-DCB, radioactivity in urine (collected for up to 175 hours after dosing) accounted for about 75, 84, and 75% of

the radioactivity for administered doses, respectively (Hissink et al. 1996a). Radioactivity in feces accounted for about 16, 12, and 7% of the respective administered doses. These results indicate absorption of at least 75–84% of the administered dose (assuming that none of fecal radioactivity was absorbed) occurred, and up to 82–96% of the dose (assuming that all radiolabel in the feces was first absorbed and later excreted in the bile) may have been absorbed. Rapid absorption was indicated since peak levels of radioactivity in blood samples occurred at about 6, 10, and 24 hours after administration of 5, 50, and 250 mg/kg doses, respectively (Hissink et al. 1996a). Other studies have identified the presence of metabolites of 1,2-DCB in the urine following oral exposure (Azouz et al. 1955; Hissink et al. 1996c).

1,3-Dichlorobenzene. Quantitative data on the absorption of 1,3-DCB in humans following oral exposure are not available. However, evidence for absorption of 1,3-DCB in humans comes from studies that have detected 1,3-DCB in breast milk (Mes et al. 1986), blood (Bristol et al. 1982), and adipose tissue (Jan 1983). While these studies do not provide a quantitative measure of the rate or extent of 1,3-DCB and cannot provide information concerning possible exposure route, they provide evidence of 1,3-DCB absorption in humans.

Evidence for absorption of 1,3-DCB following oral exposure of animals comes from the detection of metabolites in the urine and bile. Kimura et al. (1992) identified at least 12 metabolites in the bile of rats given 1,3-DCB by gavage, indicating that absorption and transport to the liver had occurred. In rabbits given oral 1,3-DCB, glucuronide, sulfur esters, mercapturic acid, and catechol metabolites were identified in the urine (Parke and Williams 1955), and suggested that 50–75% of the compound was absorbed, based on the presence of these metabolites.

1,4-Dichlorobenzene. Quantitative data on the absorption of 1,4-DCB in humans following oral exposure are not available. However, evidence for absorption of 1,4-DCB in humans comes from numerous studies that have detected 1,4-DCB in human tissues, including the blood (Bristol et al. 1982; Hill et al. 1995), urine (Hill et al. 1995; Ghittori et al. 1985; Pagnotto and Walkley 1965), adipose tissue (Jan 1983), and breast milk (Jan 1983). While these studies do not provide a quantitative measure of the rate or extent of 1,4-DCB and cannot provide information concerning possible exposure route, they provide evidence that 1,4-DCB is absorbed by humans.

Evidence for absorption of 1,4-DCB in animals includes studies demonstrating toxicity following oral exposure (see Section 3.2), as well as studies demonstrating the presence of 1,4-DCB or metabolites in

peripheral tissues following one or more oral exposures that indicate that 1,4-DCB is rapidly and nearly completely absorbed. Following a single or multiple oral exposures of radiolabeled 1,4-DCB in rats, label was detected in all evaluated tissues (liver, kidneys, lungs, muscle, fat, and blood plasma), indicating that considerable absorption had occurred (Hawkins et al. 1980). Additional support for a near-complete absorption comes from data showing that levels in tissues were similar following 10 oral exposures or 10 subcutaneous injections of 250 mg/kg. Levels of label in tissues did not appreciably increase with increasing the number of exposures beyond one (Hawkins et al. 1980). Similarly, Hissink et al. (1996b) reported that 70–85% of a single radiolabeled dose of 1,4-DCB was eliminated in the urine within 72 hours of exposure, indicating that 1,4-DCB was rapidly and extensively absorbed. By contrast, Klos and Dekant (1994) reported that ~41% of a labeled oral dose of 1,4-DCB was recovered in the urine 72 hours postexposure.

3.4.1.3 Dermal Exposure

1,2-Dichlorobenzene. Studies examining the absorption of 1,2-DCB in humans or animals following dermal exposure are not available.

1,3-Dichlorobenzene. Studies examining the absorption of 1,3-DCB in humans or animals following dermal exposure are not available.

1,4-Dichlorobenzene. No studies were located that specifically address the rate or amount of absorption of 1,4-DCB by humans or animals after dermal exposure to 1,4-DCB. Solid 1,4-DCB produces a burning sensation when held closely to the skin for an excessive period of time, but it does not produce irritation or systemic effects (Hollingsworth et al. 1956). In a study of the acute dermal toxicity of 1,4-DCB in adult Sherman rats, the dermal LD_{50} was estimated to be >6,000 mg/kg/day in both sexes (Gaines and Linder 1986). These data do not indicate that 1,4-DCB is absorbed to any extent after dermal exposure; dermal exposure to 1,4-DCB is associated with low systemic toxicity in both humans and laboratory animals.

3.4.2 Distribution

1,2-Dichlorobenzene. Quantitative data on the distribution of 1,2-DCB in humans are not available. 1,2-DCB has been detected in the blood (Bristol et al. 1982), urine (Kumagai and Matsunaga 1995, 1997; Zenser et al. 1997), and breast milk (Jan 1983; Mes et al. 1986) of humans.

The most comprehensive animal study of the distribution of 1,2-DCB following a single oral administration (10 mg/kg) is the study of Hissink et al. (1996a), which followed the distribution of the compound in exposed rats for up to 75 hours in 19 tissues, as well as the residual carcass and gastrointestinal tract. The results are presented in Table 3-8. 1,2-DCB was detected in all evaluated tissues, but at greatest concentrations in the urinary bladder, kidney, fat, and liver. Retention half-times ranged from 8.7 hours (urinary bladder) to 19.3 hours (brain), with only small levels of activity detectable in any tissue at 75 hours postexposure. In a separate study in the same manuscript, approximately 60% of an oral dose was found in the bile, indicating that considerable enterohepatic circulation occurs.

Twenty-two hours after a single intraperitoneal injection in Wistar rats or BALB/c mice, 1,2-DCB was found covalently bound to DNA, RNA, and proteins of liver, kidney, lung, and stomach (Colacci et al. 1990).

1,3-Dichlorobenzene. Quantitative data on the distribution of 1,3-DCB in humans are not available. However, 1,3-DCB has been detected in breast milk (Mes et al. 1986), blood (Bristol et al. 1982), and adipose tissue (Jan 1983), suggesting a wide distribution throughout the body.

Data are not available on the distribution of 1,3-DCB following inhalation exposure in animals. Kimura et al. (1983) reported the presence of 1,3-DCB or metabolites in the liver and kidney following oral exposure. Following oral exposure, 1,3-DCB undergoes enterohepatic circulation, as demonstrated by the data of Kimura et al. (1992), who identified at least 12 biliary metabolites in rats exposed to 1,3-DCB by gavage.

1,4-Dichlorobenzene. Quantitative data on the distribution of 1,4-DCB in humans are not available. However, 1,4-DCB has been detected in the blood (Bristol et al. 1982; Hill et al. 1995), urine (Hill et al. 1995; Ghittori et al. 1985; Pagnotto and Walkley 1965), adipose tissue (Jan 1983), and breast milk (Jan 1983) of humans, indicating distribution at least to those tissues.

Table 3-8. Tissue Concentrations (nmol/g tissue) of Radioactivity in Male Wistar Rats at Four Time Points after Oral Administration of 10 mg/kg ^{14}C-Labeled 1,2-Dichlorobenzene in Corn Oil

Tissue	6 hours	15 hours	30 hours	75 hours	$t_{1/2}$ (hours)
Liver	32.7±3.4	9.4±1.9	3.1±1.1	1.4±0.4	17.0
Kidney	132.5±107	15.7±4.8	3.8±0.7	1.5±0.4	13.1
Spleen	8.0±5.3	2.0±0.9	0.59±0.14	0.2±0.07	15.2
Pancreas	9.5±5.6	2.6±0.9	1.11±0.4	0.26±0.08	14.5
Lung	6.6±0.6	3.4±0.9	1.02±0.12	0.29±0.11	16.0
Heart	4.7±0.8	2.6±0.8	0.7±0.08	0.18±0.03	15.1
Brain	1.1±0.1	0.7±0.08	0.3±0.08	0.08±0.04	19.3
Skin	18.8±10.9	2.9±1.1	1.11±0.46	0.41±0.12	15.1
Femur	5.2±2.6	1.3±0.4	0.55±0.18	0.14±0.0	15.1
Skeletal muscle	4.7±3.1	1.3±0.6	0.45±0.2	0.09±0.04	13.5
Perirenal fat	33.4±12.1	14.0±2.6	2.18±0.3	0.18±0.03	9.4
Testis	3.6±0.8	1.9±0.4	1.13±0.9	0.2±0.07	17.2
Urinary bladder	183±121	17.3±13.6	6.6±6.4	0.32±0.04	8.7
Stomach	6.5±1.7	1.7±0.2	0.98±0.46	0.16±0.03	14.3
Small intestine	29.1±9.3	10.7±0.6	3.5±2.4	0.43±0.28	11.6
Caecum	16.4±4.8	16.7±1.1	2.8±2.2	0.27±0.07	11.1
Colon	7.5±2.2	12.0±2.4	1.4±0.9	0.20±0.07	12.0
Plasma	22.3±2.0	8.8±3.0	1.8±0.1	0.41±0.14	12.5
Red blood cells	9.2±1.0	3.4±0.6	1.6±0.4	0.57±0.22	18.8
Residual carcass	13±3%	4±2%	1±0.2%	0.3±0.07%	No data
Gastrointestinal tract contents	13±4%	15±4%	2±1%	0.1±0.04%	No data

Source: Hissink et al. 1996a

Studies in animals indicate that following absorption, 1,4-DCB is rapidly distributed throughout the body. Initially, 1,4-DCB accumulates in adipose tissue, but is not retained long-term. While distributed rapidly throughout the body, studies have demonstrated that very little of a dose of 1,4-DCB remains in tissues 72 hours postexposure (Hissink et al. 1996b; Klos and Dekant 1994; Umemura et al. 1998).

Following a single 24-hour inhalation exposure in rats, serum concentrations of 1,4-DCB rose sharply during the first 6 hours, then slowly for the next 18 hours. A sharp increase was seen in serum 1,4-DCB levels during the first 3 hours postexposure, which decreased rapidly thereafter. The greatest tissue concentrations of 1,4-DCB were found in the fat; concentrations in fat increased rapidly for the first 12 hours, then leveled off, remaining more or less steady until 6 hours postexposure, at which time they declined sharply (Umemura et al. 1990). Levels in the liver and kidney were approximately equivalent, although 10- to 20-fold lower than those in fatty tissues; in both liver and kidney, there was a steady increase in 1,4-DCB concentration for the 24 hours of exposure. In parallel with serum 1,4-DCB levels, there was a sharp, unexplained jump in the concentration of 1,4-DCB in both liver and kidney at 3 hours postexposure that resolved by 6 hours postexposure; concentrations fell rapidly thereafter. Following single or multiple inhalation exposures to radiolabeled 1,4-DCB, the greatest concentrations of label were found in the fat, with levels 10- to 20-fold greater than any other examined tissue (Hawkins et al. 1980). In nonfat tissues, the kidney showed the greatest amounts of label, on a per gram of tissue basis, followed by the liver, blood plasma, lungs, and muscle (Hawkins et al. 1980).

Following single or multiple oral exposures to radiolabeled 1,4-DCB, the greatest concentrations of label were found in the fat, with levels 6- to 15-fold greater than any other examined tissue (Hawkins et al. 1980). In nonfat tissues, the kidney showed the greatest amounts of label, on a per gram of tissue basis, followed by the liver, blood plasma, lungs, and muscle (Hawkins et al. 1980). Hissink et al. (1997a) reported that after a single oral dose of radiolabeled 1,4-DCB, a steady increase in radiolabel found in the blood, and in the plasma compartment, was seen for the first 8–10 hours, after which concentrations decreased steadily for the next 40 hours.

Within 12 hours after exposure of male rats to a single oral dose of 1,4-DCB, two sulfur-containing metabolites, 2,5-dichlorophenyl methyl sulfoxide, and 2,5-dichlorophenyl methyl sulfone (M2), were found in the blood, urine, fat, liver, and kidneys (Kimura et al. 1979). These metabolites remained in the blood after most of the 1,4-DCB had fallen below the detection limits of the assay. The maximum concentration of 2,5-dichlorophenyl methyl sulfoxide in blood was reached 15 hours after dosing and declined rapidly thereafter. For 2,5-dichlorophenyl methyl sulfone, two peaks were detected at 18 and

48 hours after dosing, which suggested to the authors that 2,5-dichlorophenyl methyl sulfone might undergo enterophepatic circulation. Changes in the levels of these metabolites in blood and tissues over a 120-hour period led the authors to suggest that 2,5-dichlorophenyl methyl sulfone might arise from 2,5-dichlorophenyl methyl sulfoxide.

3.4.3 Metabolism

Fisher et al. (1995) compared the metabolism and toxicity of the DCB isomers in liver slices prepared from human donor tissues, and from male Sprague-Dawley and F344 rats. At 2 and 6 hours, the metabolism of 1,4-DCB in human liver slices was similar to that seen in Sprague-Dawley and F344 rats. In human and F344 rat liver slices, the metabolism of 1,4-DCB was intermediate to that of 1,3- and 1,2-DCB at 2 hours; at 6 hours, the metabolism of 1,4-DCB was lower than that of 1,3- or 1,2-DCB. In Sprague-Dawley rats, the hepatic metabolism of 1,4-DCB was greater than that of 1,3- and 1,2-DCB at 2 hours, while at 6 hours, the metabolism of 1,4-DCB was intermediate to that of 1,3- or 1,2-DCB. In all three species, the metabolism of 1,4-DCB was not linear over time; the amount metabolized at 6 hours was only slightly higher than that metabolized after 2 hours. At both 2 and 6 hours, the amount of glucuronide and sulfate conjugates produced from 1,4-DCB was similar across all of the tested species.

1,2-Dichlorobenzene. The initial step in the metabolism of 1,2-DCB is metabolism by cytochrome P-450 isozymes, mainly P4502E1, to an active epoxide. This epoxide can either react directly with cellular components, be conjugated to glutathione or glucuronic acid, or be hydrolyzed to form 2,3-dichlorophenol or 3,4-dichlorophenol. The dichlorophenol metabolites can be further metabolized by conjugation with glutathione, glucuronic acid, or sulfate, or further oxidized to catechols. An additional oxidation to form dichlorohydroquinone metabolites has also been proposed.

Microsomal studies have implicated cytochrome P-450, and particularly P4502E1, as a major component of 1,2-DCB metabolism, resulting in the formation of dichlorophenols, dichlorocatechols, and dichlorohydroquinones. After exposure to 1,2-DCB in rat liver microsomes, dichlorohydroquinone metabolites>dichlorophenol metabolites>dichlorocatechol metabolites (den Besten et al. 1992). Increasing dose results in a greater formation of dichlorohydroquinone metabolites, with less dichlorophenol and dichlorocatechol metabolites, and a greater covalent binding to proteins. When 1,2-DCB was added to hepatic microsomes from animals treated with P-450 inducers, the major metabolites were dichlorophenols and dichlorohydroquinones (den Besten et al. 1992). 1,2-DCB in this system was also metabolized to a species that bound covalently with protein; addition of ascorbic acid

decreased the binding to protein by 68% (den Besten et al. 1992). Microsomes from rats and mice pretreated with benzene to induce cytochrome P-450 resulted in greater levels of metabolism of 1,2-DCB, both to soluble or covalently-bound products, than in untreated animals (Nedelcheva et al. 1998). Addition of diethyldithiocarbamate, a P-450 inhibitor, decreased 1,2-DCB metabolism by ≥90% in both normal and pretreated hepatic microsomes from rats and mice, and in normal human liver microsomes.

Addition of glutathione to the reaction mixture containing human or rat microsomes results in considerable (50–70%) formation of the glutathione-epoxide conjugate; addition of glutathione S-transferase enhances this proportion (Hissink et al. 1996c).

The metabolism of 1,2-DCB by isolated microsomes containing human cytochrome P-450 isozymes is accomplished mainly by cytochrome P4502E1 (Hissink et al. 1996a, 1996b). Incubation of 1,2-DCB with microsomes from cells expressing human cytochrome P-450 enzymes indicated that the 3,4-dichlorophenol was formed in greater amounts than the 2,3-dichlorophenol, and that in both cases, cytochrome P4502E1 was the most active isozyme (Bogaards et al. 1995).

Experiments using rat and human liver slices have detected the presence of sulfatase, glucuronide, and glutathione/cysteine conjugates following exposure to 1,2-DCB (Fisher et al. 1990, 1995). Covalent binding of 1,2-DCB metabolites to proteins has also been shown in experiments using rat and liver slices (Fisher et al. 1990, 1995).

Fisher et al. (1990) reported that in rat liver slices, the majority (>70%) of 1,2-DCB was found conjugated to glutathione, or as a cysteine conjugate, with only small amounts of the glucuronide or sulfate detected; only the conjugation status of the metabolite was reported. In human liver slices, the pattern was different, with approximately equal distribution of glucuronide and glutathione conjugates, and only minor amounts of the sulfate. Human liver slices metabolized approximately 50% more 1,2-DCB than did slices from F344 rats, and approximately 4-fold as much as slices from Sprague-Dawley rats (Fisher et al. 1995). Human liver slices formed 7–30-fold greater levels of glucuronide conjugates, 1.5–2-fold more sulphatase conjugates, and 1.5–2-fold more glutathione/cysteine conjugates of 1,2-DCB than rat liver slices (Fisher et al. 1995). Human fetal liver slices metabolized 1,2-DCB only about 10% as much as adult liver, and did so predominantly with conjugation to glutathione-S-transferase (GSH) (Fisher et al. 1990).

Azouz et al. (1955) identified urinary metabolites of 1,2-DCB in rabbits exposed to a single *in vivo* dose; 2,3- and 3,4-dichlorophenol were detected, as were considerable levels of glucuronide and sulfate conjugates; the presence of dihydroquinone metabolites was not reported. Pretreatment of F344 rats with inducers of cytochrome P-450 (phenobarbital, β-naphthoflavone, or pyridine) resulted in an increased toxicity of intraperitoneal 1,2-DCB while treatment with piperonyl butoxide, a P-450 inhibitor, reduced the toxicity of 1,2-DCB (Valentovic et al. 1993b). Evidence for binding of 1,2-DCB or its metabolites to glutathione includes the depletion of hepatic glutathione following a single intraperitoneal injection of 3.6 mmol/kg of 1,2-DCB in F344 or SD rats (Younis et al. 2000); depletion was nearly complete at 8 hours postinjection, and remained nearly complete at 12 hours postinjection. Fischer 344 rats recovered by 24 hours postinjection, but SD rats remained depleted.

Kumagai and Matsunaga (1995, 1997) reported that in occupationally-exposed humans, conjugated urinary metabolites of 1,2-DCB consisted of 3,4- and 4,5-dichlorocatechol and 2,3- and 3,4-dichlorophenol; there was a linear correlation between exposure concentration and the levels of these four metabolites in the urine.

1,3-Dichlorobenzene. Data on the metabolism of 1,3-DCB are less available than for the other two isomers of DCB. However, the available studies indicate that 1,3-DCB is metabolized by cytochrome P-450 to an epoxide and later to a dichlorophenol, followed by considerable secondary metabolism, similar to 1,2- and 1,4-DCB.

Fisher et al. (1990) reported that in rat liver slices, the majority (~70%) of 1,3-DCB was found conjugated to glutathione, or as a cysteine conjugate, with only small amounts of the glucuronide or sulfate detected. In human liver slices, the pattern was different, with approximately equal distribution (~40% each) of glucuronide and glutathione conjugates, and ~20% of the metabolites as the sulfate.

Human liver slices metabolized greater amounts of 1,3-DCB than did slices from F344 or Sprague-Dawley rats (Fisher et al. 1995). Human liver slices formed 2–9-fold greater levels of glucuronide conjugates, 1–4-fold greater levels of sulphatase conjugates, and 1–4-fold greater levels of glutathione/cysteine conjugates of 1,3-DCB than rat liver slices (Fisher et al. 1995).

Following *in vivo* exposure of rats to 1,3-DCB, the major sulfur-containing metabolites in the urine were 2,4- and 3,5-dichlorophenyl methyl sulfoxides and 3,5- and 2,4-dichlorophenyl methyl sulfones (Kimura et al. 1983). Kimura et al. (1992) identified 18 different biliary metabolites in rats exposed to a single

dose of 1,3-DCB; these were all heavily conjugated dichlorophenyl metabolites, with evidence of both mono- and diol formation, but no conjugated quinone derivatives.

Parke and Williams (1955) reported that following administration of 1,3-DCB to rabbits, the major urinary metabolites were 3,5-dichlorophenol and 2,4-dichlorophenol; the urine also contained 2,4-dichlrophenylmercapturic acid.

1,4-Dichlorobenzene. In general, the basic steps in metabolism of 1,4-DCB are similar to those of the other DCB isomers. The initial metabolic step is oxidation by cytochrome P-450, primarily P4502E1, to an epoxide and further to 2,5-dichlorophenol. The dichlorophenol may be further oxidized to dichlorocatechols, or possibly a dichlorohydroquinone, or may be conjugated by several phase II metabolism pathways. Support for the cytochrome P-450-mediated oxidation of 1,4-dichlorophenol, and subsequent conjugation reactions, comes from studies in isolated microsomes, liver slices, and exposures *in vivo*.

Analysis of the urine specimens of a 3-year-old boy who had been playing with 1,4-DCB yielded 2,5-dichlorophenol as well as four other unidentified phenols. These compounds were shown to be conjugated with glucuronic and sulfuric acids (Hallowell 1959).

After treatment of F344 rats with 1,4-DCB, the major biotransformation reaction is P-450-dependent oxidation to 2,5-dichlorophenol, which is then primarily conjugated to sulphate or glucuronic acid and eliminated in the urine (Hissink et al. 1996b; Klos and Dekant 1994); mercapturic acids were also identified in the urine of exposed rats. Following a single oral exposure of 1,4-DCB to male Wistar rats, the main sulfur-containing metabolites found in the urine were 2,5-dichlorophenyl methyl sulfoxide (M1) and 2,5-dichlorophenyl methyl sulfone (M2); levels of M2 in the blood were greater, and more persistant, following a single oral dose of 1,4-DCB (Kimura et al. 1979).

Hissink et al. (1997a) exposed male Wistar rats to 0, 10, 50, or 250 mg/kg of 1,4-DCB. Approximately 90% of the DCB was metabolized to the 2,5-dichlorophenol, which was detected in the urine as its sulfate (50–60%), glucuronide (20–30%), and the free form (5–10%); in the bile, the major metabolite was the glucuronide of 2,5-dichlorophenol. The remaining metabolites consisted of N-acetyl-cysteine-S-dihydro-hydroxy-1,4-DCB and N-acetyl-cysteine-S-1,4-DCB. No evidence for the formation of hydroquinones was seen, even under conditions of induced oxidative metabolism.

Following oral administration to Chinchilla rabbits, 1,4-DCB was also oxidized, principally to 2,5-dichlorophenol. A very high percentage of this metabolite was eliminated in the urine as conjugates of glucuronic or sulfuric acids (Azouz et al. 1955). Sulfur metabolites (methyl sulfides and methyl sulfones) of 2,5-dichlorophenol have been shown to induce cytochrome P450 activity (Kimura et al. 1983).

Fisher et al. (1990) reported that in rat liver slices, the majority (>60%) of 1,4-DCB was found conjugated to glutathione, or as a cysteine conjugate, with small amounts of the sulfate detected as well (~10% of total metabolites). In human liver slices, the pattern was different, with glutathione still being the predominant metabolite (~55%), but with an approximately equal distribution of glucuronide and sulfate conjugates (22–24%). In a later study, Fisher et al. (1995) reported that the total metabolism of 1,4-DCB was similar in liver slices from F344 rats, Sprague-Dawley rats, and humans. Human liver slices formed greater levels (~20–50%) of glucuronide conjugates of 1,4-DCB than rat liver slices; levels of formation of sulphatase and glutathione conjugates were similar in rats and humans (Fisher et al. 1995).

After a single exposure to 1,4-DCB in rat liver microsomes, dichlorohydroquinone metabolites were formed at greater levels than dichlorophenol metabolites, which in turn were more prevalent than dichlorocatechol metabolites (den Besten et al. 1992). Increasing the concentration does not change the percent formation of 2,5-dichlorohydroquinone, but decreases the formation of dichlorophenols in favor of increased covalent binding to proteins. Hissink et al. (1997b) reported that incubation of 1,4-DCB with microsomes of rat or mouse liver, in the presence of glutathione but lacking ascorbic acid or glutathione transferase enzymes, resulted primarily in the formation of S-glutathionyl-dichlorocatechol metabolites, 2,5-dichlorophenol, and 2,5-dichlorohydroquinone; rats appeared to be more efficient at forming a glutathione conjugate of the 2,3-epoxide than did mice, and formed less unconjugated 2,5-dichlorophenol and 2,5-dichlorhydroquinone.

Incubation of 1,4-DCB with microsomes from cells expressing human cytochrome P-450 enzymes indicated that the 2,5-dichlorophenol was the only isomer formed, and that cytochrome P4502E1 was the most active isozyme in its formation (Bogaards et al. 1995; Hissink et al. 1996a, 1996b). In human microsomes, metabolism of 1,4-DCB was lower than in rodents, with 2,5-dichlorophenol as the major metabolite, even in the presence of added GSH (Hissink et al. 1997b). Using cell lines expressing individual human cytochrome P-450 isozymes, it was revealed that CYP2E1, and not 1A1, 1A2, 2B6, 2C9, 2D6, 2A6, or 3A4, participated in 1,4-DCB metabolism.

Addition of diethyldithiocarbamate, a P-450 inhibitor, decreased 1,2-DCB metabolism by ≥90% in both normal or pretreated hepatic microsomes from rats and mice, and in normal human liver microsomes (Nedelcheva et al. 1998), providing additional evidence for the involvement of cytochrome P-450 in 1,4-DCB metabolism.

3.4.4 Elimination and Excretion

1,2-Dichlorobenzene. Following absorption, 1,2-DCB is eliminated primarily in the urine of both humans and animals, as metabolites rather than as the parent compound. Studies have detected the metabolites of 1,2-DCB in the urine of occupationally exposed humans (Kumagai and Matsunaga 1995, 1997; Zenser et al. 1997). While a linear correlation between airborne concentration and urinary metabolite levels has been demonstrated, a quantitative assessment of the percent urinary elimination has not been determined.

Quantitative data on elimination of 1,2-DCB comes from the study of Hissink et al. (1996a), which reported that following a single oral exposure to radiolabeled 1,2-DCB, 75–84% of the activity was detected in the urine 175 hours postexposure, with 7–16% being detected in the feces. Azouz et al. (1955) has also reported the elimination of 1,2-DCB and metabolites in the urine of exposed animals, although quantitative assessments of elimination were not presented.

1,3-Dichlorobenzene. Data on the elimination of 1,3-DCB in humans are not available.

Following a single dose of 1,3-DCB in rabbits, 50–75% of the compound was detected as urinary metabolites, indicating that the major route of elimination for 1,3-DCB is via the urine (Parke and Williams 1955). Kumura et al. (1984) also reported the presence of urinary metabolites of 1,3-DCB, although quantitative data were not presented. Additional data on the elimination of 1,3-DCB are not available.

1,4-Dichlorobenzene. Quantitative data on the elimination of 1,4-DCB in humans are not available. However, metabolites of 1,4-DCB have been detected in the urine of exposed humans (Ghittori et al. 1985; Hill et al. 1995; Pagnotto and Walkley 1965), demonstrating the urinary elimination of 1,4-DCB in humans.

Animal studies of 1,4-DCB elimination have demonstrated that the compound is eliminated mainly in the urine, regardless of exposure route; elimination occurs in the form of metabolites, rather than as the parent compound. Male Wistar rats given single oral doses of 10, 50, or 250 mg/kg of ^{14}C-1,4-DCB excreted the majority of 14C derived from 1,4-DCB in the urine as either the sulfate conjugate (60%) or the glucuronide (30%). Bile contained 5 and 30% of the total radioactivity after the low and high doses, respectively. Only minor amounts of mercapturic acid were found (Hissink et al. 1996b). In a later study, Hissink et al. (1997a) reported that following a single oral dose of 1,4-DCB in male Wistar rats, 75–85% of the dose was recovered in the urine, with only 2–5% being detected in the feces; clearance half-times did not vary with increasing dose level. Biliary excretion was dose-related, ranging from <5% at 10 mg/kg to 30% at 250 mg/kg (Hissink et al. 1997a). In male and female F344 rats administered a single dose of 900 mg/kg/day ^{14}C-1,4-DCB by gavage in corn oil, the excretion of radioactivity in the urine reached a peak in both males and females between 24 and 36 hours after dosing. Seventy-two hours after dosing, 41.3 and 3.6% of the dose was found in the urine and feces, respectively, of males; corresponding values in the urine and feces of females were 41.3 and 3.6% (Klos and Dekant 1994). Following oral or inhalation exposure in rats, levels of 1,4-DCB and its metabolites decreased only slightly over the first 8 hours postexposure in the liver, kidneys, fat, and plasma, but then fell rapidly and were nearly undetectable 120 hours after the final exposure (Hawkins et al. 1980). Elimination was primarily urinary, with 97% of the total recovered label found in the urine (Hawkins et al. 1980). Elimination in the expired air was negligible, being 1% of the total or less (Hawkins et al. 1980).

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

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

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and

Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

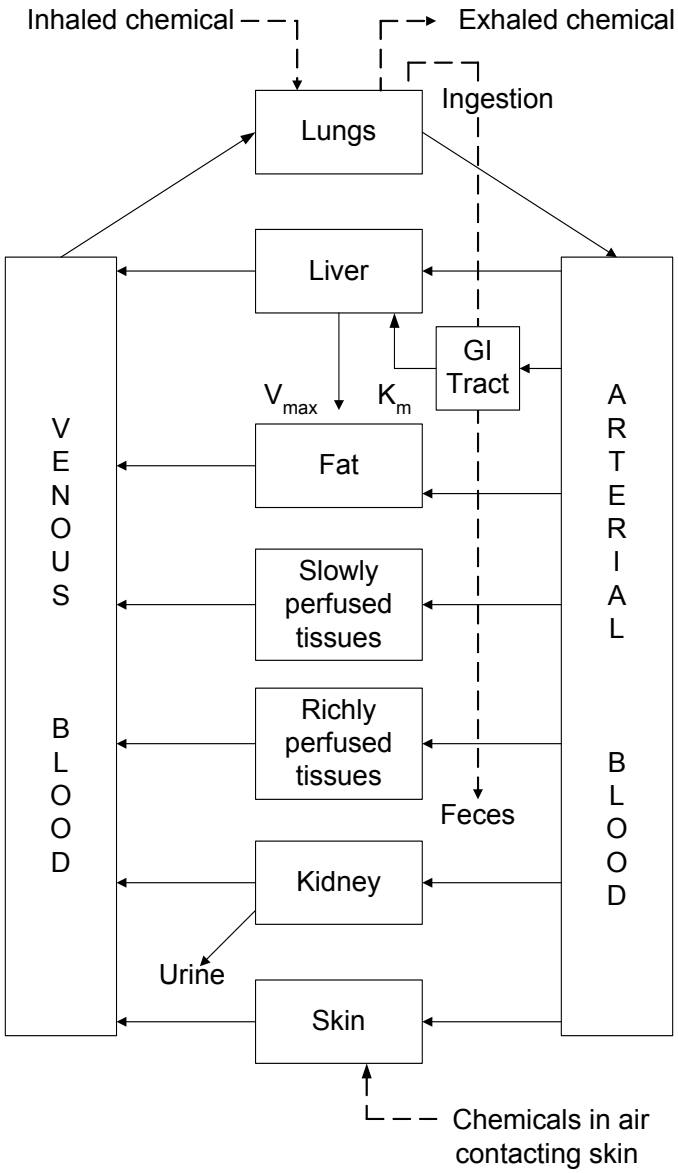
The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

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

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-6 shows a conceptualized representation of a PBPK model.

If PBPK models for dichlorobenzenes exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Figure 3-6. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

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

PBPK models are available for 1,2-DCB in rats and humans (Hissink et al. 1997b). No PBPK models have been developed for 1,3- or 1,4-DCB.

The rat and human PBPK models for 1,2-DCB were developed for oral exposure and do not include respiratory or dermal portals of entry (Hissink et al. 1997b). Both models have four compartments connected by blood flows: rapidly perfused tissues including the lung, kidneys, and spleen; slowly perfused tissues comprising muscle and skin; fat; and the liver, the only compartment in which metabolism is assumed to take place. The models assume that gastrointestinal tract uptake proceeds as a dose-dependent first-order kinetic process in which 1,2-DCB is deposited directly in the liver. For each of the nonmetabolizing compartments, differential equations describe the influx and efflux of 1,2-DCB. Equations are also used for the liver compartment to accounted for 1,2-DCB metabolism and reduced glutathione (GSH) synthesis, turnover, and consumption. Physiologic parameters, partition coefficients, biochemical parameters, and absorption rate constants used in the models are shown in Table 3-9. Absorption rate constants were estimated by fitting of the parameters to data for rats exposed to 5, 50, or 250 mg/kg 1,2-DCB.

Metabolism in the model is described as the initial, P-450-mediated, saturable formation of an epoxide, followed by epoxide transformation via three competing pathways that are assumed to independently follow pseudo first-order kinetics (i.e., are non-saturable): (1) conversion into dichlorophenol; (2) covalent binding to cellular macromolecules; and (3) conjugation with GSH. Michaelis-Menten constants, Vmax and Km, for the saturable cytochrome-P-450 oxidation of 1,2-DCB were initially estimated (in units of nmol/min-mg protein) from *in vitro* experiments with rat and human liver microsomes (Table 3-9). Scaling for use in the models assumed rat and human values of 45 and 77 mg microsomal protein/g liver, respectively. However, in order to obtain adequate fits to rat data for blood concentrations of parent material or total amount of metabolites, a "best-fit" Vmax value of 17 μmol/hour was used, along with the *in vitro* Km of 4.8 μM (Table 3-9). This "best-fit" value was about 4-fold higher than the rat *in vitro* Vmax scaled to units of μmol/hour (4.3 μmol/hour; see Table 3-9). Based on the rat data analysis, a factor of four was used to derive a "best-fit" Vmax value of 10,840 μmol/hour from the human *in vitro* Vmax (2,742 μmol/hour; see Table 3-9). The ratio of rate constants for the three epoxide-transforming pathways in rats (5:30:65) was estimated based on the relative amounts of *in vitro* covalent binding (5%), *in vitro* and *in vivo* dichlorophenol formation (25 and 30%), and *in vitro* and *in vivo* GSH conjugation (70 and 60%). For the rat model, the first-order rate constant for covalent binding was arbitrarily set at 50 $hour^{-1}$; the resultant constants for dichlorophenol formation and GSH conjugation

Table 3-9. Parameters in PBPK Models for 1,2-Dichlorobenzene

Parameter	Rat	Human
Physiologic parameters (as per Gargas et al. 1986)		
Body weight (kg)	0.258	70
Percentages of body weight		
Liver	4	3.14
Fat	7	23.1
Rapidly perfused	5	2.66
Slowly perfused	75	62.1
Flows (L/hour) [QC or QP= 15 L/hour (body weight)$^{0.74}$]		
Cardiac output (QC)	5.50	348.0
Alveolar ventilation (QP)	5.50	348.0
Percentages of cardiac output		
Liver	25	25
Fat	9	9
Rapidly perfused	51	51
Slowly perfused	15	15
Partition coefficients [calculated by methods of Droz et al. (1989) based on water:air, oil:air, and blood:air partition coefficients]		
Blood:air	423	423
Liver:blood	2.7	2.7
Fat:blood	66.4	66.4
Rapidly perfused:blood	2.7	2.7
Slowly perfused: blood	1.3	1.3
Biochemical parameters		
1,2-Dichlorobenzene oxidation		
Vmax (nmol/min-mg) (*in vitro* derived)	0.142 (4.3 µmol/hour)	0.27 (2,742 µmol/hour)
Km (µM) (*in vitro* derived)	4.8	7.5
Vmax (µmol/hour) (“best-fit” values)	17	10,840
GSH conjugation of epoxide (hour^{-1})	650	650
Formation of dichlorophenol (hour^{-1})	300	360
Formation of reactive metabolites (hour^{-1})	50	5
GSH turnover rate (hour^{-1})	0.14	0.14
Absorption rate constants (estimated by fitting parameters to data for rats at indicated dose levels)		
Ka (hour^{-1})		
5 mg/kg	0.5	No data
50 mg/kg	0.18	No data
250 mg/kg	0.06	0.06

Source: Hissink et al. 1997b

were 300 and 650 $hour^{-1}$, respectively (Table 3-9). *In vitro* data with human microsomes similarly formed the basis of the rate constants for these pathways: 5 $hour^{-1}$ for covalent binding, 360 $hour^{-1}$ for dichlorophenol formation, and 650 $hour^{-1}$ for GSH conjugation (Table 3-9). A GSH turnover rate of 0.14 $hour^{-1}$, determined in another study with rats (Potter and Tran 1993), was used in both the rat and human models (see Table 3-9).

The rat model was used to predict hepatic concentrations of covalently bound metabolites following an oral dose of 250 mg/kg 1,2-DCB that was expected to be toxic to the liver (Hissink et al. 1997b). The hepatic concentration in rats, 24 hours after dosing, was 1,459 μM. Versions of the human model using different Vmax values predicted that this administered dose level produced much lower hepatic concentrations of covalently bound metabolites in humans. Increasing the human *in vitro*-derived Vmax values by a factor of 10 did not increase the predicted human hepatic concentrations, 24 hours after dosing, to a value above about 240 μM. Therefore, the models predicted that equivalent administered doses in rats and humans would produce rat hepatic concentrations of covalently bound metabolites that are at least 6-fold higher in rats than humans.

The PBPK models were also used to predict hepatic concentrations of GSH (expressed as a percentage of an assumed baseline concentration of 6.5 mM) following an oral dose of 250 mg/kg 1,2-DCB (Hissink et al. 1997b). The rat model predicted that maximum depletion of GSH (about 70% depletion) occurred at 15 hours after dosing with 250 mg/kg. In contrast, the human model (using a Vmax value of 10,840 μmol/hour; see Table 3-9) predicted that maximum depletion of GSH (essentially 100% depletion) occurred at 10 hours after dosing. The models therefore predicted that humans may be more susceptible to 1,2-DCB depletion of hepatic GSH levels than are rats. Hissink et al. (1997b) noted that (1) if depletion of GSH is the only factor involved in acute 1,2-DCB hepatotoxicity, the models predict that humans may be more susceptible than rats at the same administered dose levels, and (2) if covalent binding of reactive metabolites is the critical factor, humans may be less susceptible to 1,2-DCB acute hepatotoxicity than rats. However, at present, the majority of parameters of the human model are based on direct scaling from the rodent data, rather than having been calibrated and validated using human data. Because the predictive ability of the human model has not been established, its usefulness is unclear.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. Quantitative inhalation, oral, or dermal absorption studies in humans are not available for 1,4-DCB. In the few studies available in laboratory animals, absorption was demonstrated to occur during a 3-hour inhalation exposure to 1,000 ppm of 1,4-DCB (Hawkins et al. 1980) as evidenced by accumulation of ^{14}C in liver, kidney, plasma, and adipose tissue. No studies were located that described the absorption characteristics of 1,4-DCB after oral exposure; however, given the structural and physicochemical similarity to benzene, oral absorption is thought to be at or near 100% (EPA 1987a; Hawkins et al. 1980). A study assessing dermal absorption reported a dermal LD_{50} of >6,000 mg/kg/day in rats (Gaines and Linder 1986). Given the physicochemical properties, similarity to benzene, and lipid-soluble properties of 1,4-DCB, absorption by the inhalation, oral, and dermal routes of exposure is most likely by simple diffusion across cellular lipid membranes. No information is available that describes site-specific absorption within the respiratory tract (nasal epithelial absorption as opposed to alveolar absorption) or in the gastrointestinal tract.

Distribution. Quantitative inhalation, oral, or dermal distribution studies in humans are not available for 1,4-DCB. 1,4-DCB has been detected in human blood, adipose tissue, and breast milk after an assumed inhalation exposure in Tokyo residents (Morita and Ohi 1975; Morita et al. 1975), as well as people in some parts of the United States (EPA 1983b, 1986f). The available data indicate that after inhalation, oral, and subcutaneous exposure, 1,4-DCB preferentially distributes to the fat tissue and organ-specific sites within the body (Hawkins et al. 1980), following the order: adipose > kidney > liver > blood (Charbonneau et al. 1989b; Hawkins et al. 1980). Although 1,4-DCB is originally distributed primarily to adipose tissue, significant amounts of 1,4-DCB are not retained in that tissue after exposure ceases. Regardless of exposure route, most of the 1,4-DCB falls to near- or below-detectable assay limits in all tissues of the body except adipose tissues 48–72 hours after exposure, depending on the dose (Charbonneau et al. 1989b; Kimura et al. 1979). 1,4-DCB was detected in adipose tissue at 120 hours after exposure (Charbonneau et al. 1989b). In the kidney, 50% of the 1,4-DCB appears to localize within the cytosol in male F344 rats (Charbonneau et al. 1987). 1,4-DCB also does not appear to bind to tissue proteins (Klos and Dekant 1994).

Metabolism/Excretion. Quantitative inhalation, oral, or dermal metabolism and excretion studies in humans are not available for 1,4-DCB. One case study involving a 3-year-old boy who may have ingested 1,4-DCB reported the presence of 2,5-dichlorophenol in the urine (Hallowell 1959). Several

laboratory animal studies have indicated that 1,4-DCB is metabolized by phase I metabolism to 2,5-dichlorophenol (probably by cytochrome P-450), which then undergoes phase II metabolism/conjugation to the glucuronide or sulfate (Azouz et al. 1955; Hawkins et al. 1980; Hissink et al. 1996a; Kimura et al. 1979; Klos and Dekant 1994). Minor amounts of 2,4-dichlorohydroquinone may also be present (Klos and Dekant 1994). Metabolism occurs in the liver. None of the detected metabolites have been reported to be associated with the toxic effects seen with 1,4-DCB. Metabolites are excreted mostly in the urine (Azouz et al. 1955; Hissink et al. 1996a; Kimura et al. 1979); however, some metabolites (mainly the glucuronide conjugate) may also be excreted in the bile and feces (Hissink et al. 1996a). The role of enterohepatic circulation in the metabolism and excretion of metabolites is not completely known; however, it has been suggested that enterohepatic circulation may occur with some sulfated metabolites (Kimura et al. 1979). This phase I and II metabolic pathway mechanism (see below) seems plausible, in that other chemicals with similar (halogenated- and lipid-soluble) physicochemical properties undergo very similar metabolic routines to become more water-soluble and excreted. The data suggest that metabolism and excretion are similar in several species. It is likely that human metabolic pathways are similar, if not identical, to those established in laboratory animals.

3.5.2 Mechanisms of Toxicity

The precise mechanism of 1,4-DCB oxidation to 2,5-dichlorophenol has not thoroughly been investigated. 1,4-DCB is known to be metabolized by cytochrome P-450 (Azouz et al. 1955; Hawkins et al. 1980) in order to be presented to phase II metabolic pathways to increase its water solubility for excretion. A proposed metabolic pathway involving cytochrome P-450 with intermediate formations of metabolites has been outlined for 1,4-DCB (Den Besten et al. 1992). No information was available regarding specific or altered mechanisms of action for 1,4-DCB in children. The hepatotoxicity and nephrotoxicity observed in laboratory animals are likely due to the formation of toxic intermediates formed while converting 1,4-DCB to 2,5-dichlorophenol by cytochrome P-450, or by depletion of GSH at higher doses of 1,4-DCB, or both. Some indirect evidence of this was provided by Mizutani et al. (1994). In mice pretreated with DL-buthionine sulfoximine (BSO), a glutathione synthesis inhibitor, a single dose of 300 mg/kg 1,4-DCB caused significant elevations of ALT and liver calcium, both peaking between 24 and 32 hours after dosing and declining thereafter, indicative of hepatic damage. Necrotic changes were observed at those times as well as hemorrhage, fatty changes, and appearance of altered eosinophilic cells. A single 1,200 mg/kg dose of 1,4-DCB did not significantly alter ALT or liver calcium, but doses of 100 mg/kg or higher in mice pretreated with BSO produced dose-related alterations in these parameters. Increasing cellular GSH with GSH monoethyl ester protected the liver from the combination

of 1,4-DCB and BSO. In addition, pretreatment with microsomal cytochrome P-450-dependent monooxygenase inhibitors also protected the liver from the combined toxicity of 1,4-DCB and BSO. Pretreatment with the P-450 inducer beta-naphthoflavone did not significantly alter the effect of 1,4-DCB plus BSO. Pretreatment with phenobarbital partially blocked the effect of 1,4-DCB plus BSO on ALT and completely prevented the increase in liver calcium. PCBs prevented the effect on both ALT and liver calcium. Treatment with BSO alone or in combination with 1,4-DCB (300 mg/kg) greatly decreased hepatic GSH concentration, the effect being more pronounced with the combination. 1,4-DCB alone had no such effect. Depletion of GSH also has been reported to increase the toxicity of 1,4-DCB in rats (Stine et al. 1991). The data provide a strong indication that the mechanism behind the hepatic (and probably renal) toxicity of 1,4-DCB lies in the intermediate steps of metabolite formation and conjugation by cytochrome P-450. Formation of 2,5-dichlorophenol from 1,4-DCB via cytochrome P-450 metabolism likely produces some intracellular, intermediate metabolite(s) that are also hepatotoxic when sufficient amounts accumulate intracellularly. These yet unidentified metabolites are detoxified by GSH, but when GSH depletion occurs, which is likely to occur at higher oral doses, toxicity is enhanced. Hepatocytes respond to these insults by releasing intracellular enzymes (Carlson and Tardiff 1976; Umemura et al. 1996), degeneration, vacuolation (Eldridge et al. 1992; NTP 1987; Rimington and Ziegler 1963), necrosis, and increases in gross liver weight (Hollingsworth et al. 1956; Riley et al. 1980a). However, these changes are not specific to 1,4-DCB and likely occur in a dose-responsive manner. At lower doses, cellular proliferation in the liver in the absence of these toxic-type responses has been observed (Eldridge et al. 1992; Umemura et al. 1996); however, the mechanism behind this response needs to be more clearly defined. Exposure to 1,4-DCB likely follows similar metabolic pathways in the kidneys and would be responsible for the toxicity (increased organ weight, tubular degeneration, nephropathy) observed in that organ, and may also be linked to the known formation of cancer-linked $\alpha_{2\mu}$-globulin in male rats.

The metabolism of 1,4-DCB could involve the formation of an arene oxide intermediate, as has been proposed to occur in the oxidative metabolism of many halogenated aromatic hydrocarbons (Jerina and Daly 1974). 1,4-DCB has not been shown to be mutagenic in microbial or mammalian systems, a result that may be viewed as further suggestive evidence that an arene oxide intermediate is not involved in its metabolism.

1,4-DCB has also been reported to produce hematological effects associated with exposure in humans and laboratory animals. These findings have been limited to red and white blood cell anomalies (NTP 1987) in rats and mice, and may take place within the bone marrow at the time of red and white cell formation, although a precise and careful mechanism behind this finding has not been produced. Acute hemolytic

anemia and methemoglobinemia reportedly occurred in a 3-year-old boy who had played with, and possibly ingested, 1,4-DCB crystals (Hallowell 1959). A 21-year-old pregnant woman who had eaten 1–2 blocks of 1,4-DCB toilet air freshener per week throughout pregnancy developed severe microcytic, hypochromic anemia with excessive polychromasia and marginal nuclear hypersegmentation of the neutrophils. Heinz bodies were seen in a small number of the red cells. After she discontinued this practice (at about 38 weeks of gestation), her hemoglobin levels began to rise steadily. The mechanism behind these findings in the human exposures is unknown, but it appears that 1,4-DCB may have some local effect on the hemoglobin content of the red blood cell (hemolysis, methemoglobinemia, Heinz bodies). These are rare events in humans and only occur at very high exposure doses in laboratory animals. The clinical finding of Heinz-body formation in red blood cells and methemoglobinemia suggest that some form of oxidative stress is occurring to produce these findings, although the mechanisms behind these end points are not known. While there may not be any direct evidence, it is not unreasonable to suspect that oxidant metabolites of 1,4-DCB may inhibit glucose-6-phosphate dehydrogenase (G6PD), as do metabolites of aniline, leading to Heinz body production, methemoglobinemia, and hemolysis (Trieff et al. 1993). The effect on the red and white blood cell production processes in the bone marrow (anemia, polychromasia) is quite likely an effect related to blood loss associated with bleeding from esophageal varices which form secondary to liver cirrhosis.

3.5.3 Animal-to-Human Extrapolations

No studies were identified that specifically addressed the use of animal data applied to human exposure issues specifically related to 1,4-DCB. No physiologically based pharmacokinetic models are available to estimate risk associated with human exposure to 1,4-DCB. It is difficult to compare the toxicity of 1,4-DCB in laboratory animals to the toxicity observed in humans, since little reliable human data are available for examination (see Section 3.2). From the little data available, it appears that humans do have the potential to exhibit the same toxicological features of 1,4-DCB toxicosis as demonstrated or observed in the laboratory animal models studied. Although the mechanisms have not been outlined, human hematological responses (Campbell and Davidson 1970) and liver responses (Hallowell 1959) to 1,4-DCB have been similar to the responses of laboratory animals tested (Hollingsworth et al. 1956; NTP 1987). (However, the human hematological responses were vague and quite possibly unrelated.) Although the data are not sufficient to make direct comparisons, the possibility strongly exists that human responses may be similar to those of laboratory animals, and animal data should be taken into consideration until better human data become available. With the exception of the $\alpha_{2\mu}$-globulin observation in the male rat kidney (Bomhard et al. 1988), all of the detoxication pathways present in the

laboratory animal models are present in humans. This means that humans are likely to detoxify 1,4-DCB in a similar or identical manner to that of the laboratory animals, and suggests that humans are susceptible to the liver and possibly the renal lesions outlined for the laboratory animals studied (see Section 3.5.2). Due to the lack of acceptable dosing and exposure data in humans, it is not possible at present to definitively determine the magnitude of these human toxicological responses, the dose-response relationship, or whether humans are more or less susceptible to these effects on a mg/kg/day (oral and dermal) or ppm (inhalation) basis. It is also unknown whether the sex predilection found in male rats to 1,4-DCB renal or endocrine toxicity occurs in the human male.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992) and again by Colborn et al. (1993), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997c). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As

a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Concern has been raised that many industrial chemicals, including DCBs, are endocrine-active compounds capable of having widespread effects on humans and wildlife (Colborn et al. 1993; Crisp et al. 1998; Daston et al. 1997; Safe and Zacharewski 1997; Versonnen et al. 2003). Particular attention has been paid to the possibility of these compounds mimicking or antagonizing the action of estrogen. Estrogen influences the growth, differentiation, and functioning of many target tissues, including female and male reproductive systems, such as mammary gland, uterus, vagina, ovary, testes, epididymis, and prostate. Most estrogenic chemicals have a ring structure included in the molecule, and *para*-substituted phenols generally bind better to the estrogen receptor and are more likely to exert xenoestrogenic effects than *ortho*- or *meta*-substituted compounds. In addition, there is evidence that some of these chemicals alter the thyroid hormone system, which is an important system for normal structural and functional development of sexual organs and the brain.

Insufficient information is available to adequately assess the endocrine disruptor potential of DCBs. Testing of 1,2-, 1,3-, and 1,4-DCB in the *in vitro* yeast estrogen screen (YES) assay showed that the 1,3- and 1,4- isomers were active in a concentration-responsive manner, although estrogenic potency was extremely weak (Versonnen et al. 2003). The relative potency relative to 17β-estradiol was 1.04×10^{-8} for 1,3-DCB and 2.2×10^{-7} for 1,4-DCB. The negative results for 1,2-DCB in this system are consistent with a lack of estrogenic activity of 1,2-DCB in *in vitro* yeast two-hybrid assays (Eguchi et al. 2003; Nishihara et al. 2000). The *in vivo* estrogenic activity of 1,2-, 1,3-, and 1,4-DCB was tested by measuring plasma vitellogenin (VTG) production in zebrafish (*Danio rerio*) that were exposed to each isomer for 14 days (Versonnen et al. 2003). VTG is a yolk protein precursor in teleosts and other oviparous vertebrates that is synthesized in response to estradiol stimulation. Elevated VTG levels were found in fish exposed to ≥10 mg/L of 1,4-DCB, but estrogenic potency was weak in comparison to ethynylestradiol, which increased VTG at ≥5 ng/L.

Histopathological changes occurred in the thyroid and pituitary glands of rats orally exposed to 1,3-DCB for 90 days (McCauley et al. 1995). Effects in the thyroid occurred at ≥9 mg/kg/day, the lowest tested dose, and included depletion of colloid density, characterized by decreased follicular size with scant colloid and follicles lined by cells that were cuboidal to columnar. Effects in the pituitary occurred at ≥147 mg/kg/day and included cytoplasmic vacuolization of the *pars distalis*. Increases in serum

cholesterol and serum calcium also occurred and were also believed to be related to effects on endocrine end points, possibly reflecting a disruption of hormonal feedback mechanisms, or target organ effects on the pituitary, hypothalamus, and/or other endocrine organs. Histopathological changes in endocrine tissues were not observed in intermediate- and chronic-duration studies of 1,2-DCB (NTP 1985; Robinson et al. 1991) or 1,4-DCB (Aiso et al. 2005b; Japan Bioassay Research Center 1995; Naylor and Stout 1996; NTP 1987) in rats, mice, or dogs. Measurements of thyroid and other endocrine hormones have not been conducted in any study of DCBs.

Effects of 1,2- and 1,3-DCB on reproductive function have not been investigated. There were no effects on fertility or mating in 2-generation studies of 1,4-DCB in rats exposed orally to ≤270 mg/kg/day (Bornatowicz et al. 1994) or by inhalation to ≤211 ppm (Tyl and Neeper-Bradley 1989). No adverse histopathological changes in reproductive tissues were observed in intermediate- and chronic-duration oral studies of 1,2-DCB (NTP 1985; Robinson et al. 1991), 1,3-DCB (McCauley et al. 1995), and 1,4-DCB (Naylor and Stout 1996; NTP 1987).

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics

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

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

There is little credible scientific information available on the susceptibility and toxicological effects of 1,4-DCB in children. The risk for exposure is apparently high. A study by Hill et al. (1995) measured blood levels of 1,4-DCB and urine levels of its metabolites in 1,000 adults, finding that exposure to 1,4-DCB was widespread, with 98% of the adults having measurable concentrations of 1,4-DCB metabolites in their urine. There is no evidence to indicate that children are likely to be exposed to lower amounts of 1,4-DCB from everyday living, suggesting that children are perhaps equally at risk for exposure and potential toxic side-effects.

Some information on possible health effects of DCBs in children is available from two case reports of 1,4-DCB exposure. Campbell and Davidson (1970) reported a case of a 21-year-old woman eating 1–2 toilet air-freshener blocks per week while pregnant. The mother developed hematological aberrations (hypochromic, microcytic anemia, polychromasia); however, she delivered an apparently normal female infant with no apparent hematological problems. Another report describes a 3-year-old boy who had been playing with crystals containing 1,4-DCB for 4–5 days before being admitted to the hospital. On admission, the boy was jaundiced, his mucous membranes were pale, and he was diagnosed with anemia and methemoglobinemia. After a blood transfusion, the child gradually improved, but it was unclear whether the boy actually ingested any of the 1,4-DCB (Hallowell 1959). These case reports are consistent with an expectation that health effects in children and adults are similar. Although there are no known differences in the toxicity of DCBs between adults and children, there is no evidence to substantiate the presumption.

Information on the reproductive toxicity of DCBs is essentially limited to a 2-generation oral study of 1,4-DCB in rats (Bornatowicz et al. 1994). There were no effects on mating or fertility in either generation, as assessed by a minimal number of end points (duration between mating and successful copulation and fertility index). The is a report of morphologically abnormal sperm in rats exposed to a high dose of 1,4-DCB by intraperitoneal injection (Murthy et al. 1987), but there are no studies that investigated transgenerational effects of exposure to DCBs.

Information on the developmental toxicity of 1,2-, 1,3-, and 1,4-DCB is available from oral and inhalation studies in rats and rabbits (Bio/dynamics 1989; Bornatowicz et al. 1994; Giavini et al. 1986; Hayes et al. 1985; Hodge et al. 1977; Ruddick et al. 1983; Tyl and Neeper-Bradley 1989). These studies provide no indications that DCBs are teratogenic, although fetotoxicity occurred at exposure levels that were also maternally toxic. A multigeneration study in rats that were orally exposed to 1,4-DCB found toxic effects in the pups during the nursing period, including increased neonatal mortality, dermal effects and other clinical manifestations, and reduced neurobehavioral performance (Bornatowicz et al. 1994). The postnatal developmental toxicity occurred at dose levels that were not maternally toxic and below those causing systemic toxicity in other animal studies. The results of this study indicate that postnatal developmental toxicity is the most sensitive end point in animals, and suggest a basis for potential concern in exposed children. Effects of DCBs on the immune and endocrine systems have not been adequately studied.

No studies are available that describe potential differences in the toxicokinetics or the mechanism of action of 1,4-DCB in children. No data are available that specifically describe whether 1,4-DCB or its major metabolites will cross the placenta; however, all three DCB isomers have been detected in placental tissues (Erickson et al. 1980; Pellizzari et al. 1982; Reichrtova et al. 1999). Because 1,4-DCB is not known to be genotoxic, it poses no threat to the DNA in parental germ cells. No PBPK models are available for children, fetuses/pregnant women, or infants/lactating women exposed to 1,4-DCB.

As discussed in Section 3.4, Toxicokinetics, the specific toxicokinetic behavior of 1,4-DCB in children (and immature laboratory animals) has not been reported. Based on its physicochemical properties, it is anticipated that the absorption, distribution, metabolism, and excretion of 1,4-DCB and its metabolites would be quite similar to that of the adult human (or animal), even when taking into account differences in body weight, total body water, body fat, volumes of distribution (V_D), and perhaps lower activities of some metabolizing enzymes (cytochrome P-450) during the natal and neonatal periods. 1,4-DCB is a lipid-soluble toxicant and is likely to pass across the placental membranes. It will likely accumulate in many of the same tissues in the fetus that it would normally be expected to accumulate in the adult, with the possible exception of fat storage in the fetus (Li et al. 1995). Some amount of 1,4-DCB accumulates in human breast milk (EPA 1983b), given its high lipid (milk fat) content, thereby providing a potential route of exposure to a nursing child, although there is no concrete data to support this relay exposure hypothesis. Some studies have noted that 1,4-DCB will preferentially distribute to adipose tissues in relatively high amounts, compared to accumulations in the liver and kidneys (Charbonneau et al. 1989b; Hawkins et al. 1980; Klos and Dekant 1994). Loss of maternal body fat may potentially mobilize 1,4-DCB from fat storage deposits in exposed mothers. This mobilization could result in increased blood levels and/or excretion of 1,4-DCB and its metabolites from the mother, as well as redistribution to other fat deposition sites, such as the high fat content found in breast milk.

No studies have described the interactions of 1,4-DCB with other chemicals in children, or the means by which to reduce peak absorption of 1,4-DCB after exposure.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, 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 dichlorobenzenes are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by dichlorobenzenes are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Dichlorobenzenes

Exposure to DCBs can be identified by measuring levels of the isomers in blood (Bristol et al. 1982; Hill et al. 1995; Jan 1983; Langhorst and Nestrick 1979; Pellizzari et al. 1985), urine (Ghittori et al. 1985; Hill

et al. 1995; Kumagai and Matsunaga 1995, 1997; Zenser et al. 1997), adipose tissue (Jan 1983), and breast milk (Jan 1983; Mes et al. 1986). Toxicokinetic studies (Section 3.4) indicate that DCBs are present in blood for a limited time after exposure and eliminated from the body over a period of several days, primarily in the urine as metabolites (Hissink et al. 1996a, 1996b; Kimura et al. 1979; Parke and Williams 1955). Measurement of urinary metabolites is likely to provide a better indication of recent exposure than blood or other measurements since DCBs can be excreted for several days post-exposure (Hallowell 1959). Urinary 2,5-dichlorophenol is a well-documented biomarker for monitoring worker exposure to 1,4-DCB (McKinney et al. 1970; Pagnotto and Walkley 1965). Urinary 2,3- and 3,4-dichlorophenols, as well as 3,4- and 4,5-dichlorocatechols, have been shown to be useful indicators of exposure to 1,2-DCB (Kumagai and Matsunaga 1997). Because the basic steps in the metabolism of the three DCB isomers are similar, likely biomarkers of exposure to 1,3-DCB include 2,4- and 3,5-dichlorophenols (Kimura et al. 1992). The presence of a DCB isomer and/or its conjugates in urine is not completely specific for exposure to the DCB. For example, several chlorophenols, including 2,5-dichlorophenol, have been identified as metabolites of lindane in laboratory animals. Because DCBs tend to accumulate in fat, measurements of adipose levels of the parent isomers are likely to provide useful information on long-term exposures (Jan 1983; Morita et al. 1975). There are currently no data available to assess a potential correlation between the values obtained with these measurements and the toxic effects observed in humans or laboratory animal species. Information on the analytical methods commonly used to detect and quantify 1,4-DCB in biological samples is presented in Section 6.1.

No information is available describing specific biomarkers of exposure to 1,4-DCB in children.

3.8.2 Biomarkers Used to Characterize Effects Caused by Dichlorobenzenes

There are no known specific biomarkers of effects for 1,2-, 1,3-, or 1,4-DCB because none of the health effects identified in humans or animals appear to be uniquely associated with exposure to any isomer. Biomarkers of effects for DCBs are likely to be common to the general class of halogenated aromatic hydrocarbons because DCBs and other structurally similar chemicals cause generally similar effects. For example, DCBs and other chlorinated aromatics induce a similar spectrum of hepatic effects ranging from liver enlargement and increased microsomal enzyme activities at lower levels of exposure to degenerative lesions at higher doses.

It is well documented that 1,4-DCB induces hyaline droplet formation and tubular degeneration in the kidneys of male rats at moderate-to-high levels of oral exposure. Saito et al. (1996) studied the effect of

oral treatment with 1,4-DCB on the urinary excretion of kidney-type $\alpha_{2\mu}$-globulin (aG-K) in male Sprague-Dawley rats. Groups of 3 rats received placebo or 1,4-DCB (1.5 mmol/kg/day; 220 mg/kg/day) by gavage in corn oil for 7 days. Concentrations of aG-K in the urine of 1,4-DCB-treated rats ranged from 0.04 to 0.18 mg/mL; urine concentrations increased steadily throughout the study. In contrast, aG-K concentrations were undetectable in the urine of controls at all time points. The mean concentration of aG-K in the kidneys of rats treated with 1,4-DCB was 1.15 mg/mg of soluble protein, compared to 0.35 mg/mg protein in the control group. The authors concluded that measurement of urinary aG-K would be a good indicator of 1,4-DCB exposure; however, this response is neither unique to 1,4-DCB nor applicable to human exposure cases. As discussed earlier in Section 2.5, this particular protein is produced in large amounts by male rats, accounting for 26% of their total urinary protein, but not in human males, where it was found to be present at 1% of the amount measured in male rats (Olson et al. 1990). Also, this protein is produced in only minimal quantities by females of any species or the males of other laboratory species including mice (EPA 1991i). These observations have led to suggestions that humans are probably not at risk for the type of nephropathy induced by 1,4-DCB in male rats, and that the $\alpha_{2\mu}$-globulin biomarker is inappropriate to use in humans (EPA 1991i).

No information was available describing specific biomarkers of effect in children to 1,4-DCB.

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects, see OTA (1990).

3.9 INTERACTIONS WITH OTHER CHEMICALS

Little information is available regarding possible interactions of 1,2-, 1,3-, or 1,4-DCB with other chemicals. Because DCBs are liver toxins, they might interact with other chemicals that are liver toxicants. These toxicants are many, and include ethanol, halogenated hydrocarbons (chloroform, carbon tetrachloride, etc.), benzene, and other haloalkanes and haloalkenes. DCB hepatotoxicity could also be exacerbated by concurrent exposure to acetaminophen, heavy metals (copper, iron, arsenic), aflatoxins, pyrrolizidine alkaloids (from some types of plants), high levels of vitamin A, and hepatitis viruses. Such interactions are likely to be additive or synergistic. One study found that pretreatment with DCB increased LD_{50} values for parathion in mice (EPA 1985a).

Regarding the effect of 1,4-DCB on hemolysis and formation of Heinz bodies, methemaglobinemia, and hemolytic anemia, it is likely that additive or synergistic interaction would occur with other oxidants, such as aniline and acrolein, which are known to inhibit G6PD. A human case study reported a possible interactive effect between DCB and naphthalene in a woman who developed aplastic anemia (EPA 1985a).

Perinatal evaluations were performed in offspring of female Wistar rats were exposed to diets containing 25 ppm 1,4-DCB (estimated dose 2 mg/kg/day) alone or combined with 125 ppm *p,p'*-dichlorodiphenyl-dichloroethylene (*p,p*'-DDE) from Gd 1 to Pnd 21 for a total of 42 days (Makita 2005). There were no maternal effects in either group as shown by clinical signs or changes in body weight and food consumption. Perinatal evaluations showed no gross external malformations or effects on litter size, sex ratio, or pup viability on Pnd 1 in either group. Assessments of the offspring until 6 weeks of age showed no postnatal effects on body weight gain, anogenital distance, times of eye and vaginal opening and preputial separation, or serum levels of reproductive hormones (LH and FSH in both sexes and testosterone in males at 6 weeks) in either group. Examination of the liver, kidneys, spleen, thymus, testes, epididymides, prostate, seminal vesicles, ovaries, uterus, and thymus at 6 weeks showed no effects on organ weight or histology in either group, except for increased absolute thymus weight (approximately 20% higher than controls) in female pups exposed to 1,4-DCB alone. The biological significance of this effect is unclear because it did not occur in the male offspring and was not accompanied by any histological changes. There was no effect on thymus weight or histology in male or female pups exposed to the mixture of 1,4-DCB and *p,p*'-DDE.

No information was located on interactions between DCBs and other chemicals in children.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

No population has been identified as exhibiting an unusual susceptibility to the effects of exposure to 1,4-DCB. However, based on data from studies in humans and animals, individuals with compromised liver function, infants and children with immature liver function (Hallowell 1959), and elderly people (Cotter 1953; Nalbandian and Pearce 1965) may be more at risk than the general population. Individuals having a genetic susceptibility to methemoglobin formation (such as those individuals with a deficiency of G6PD in their red blood cells) may also be at increased risk from inhalation or oral exposure to 1,4-DCB.

No information was available describing specific susceptibilities of children to 1,4-DCB. There is no direct evidence that children differ in their susceptibility to the health effects of 1,4-DCB from adults. It should be noted that postnatal neurodevelopmental toxicity is a sensitive end point in 1,4-DCB-exposed rats (Bornatowicz et al. 1994), suggesting a basis for potential concern in exposed children. This issue is discussed in detail in Section 3.7 Children's Susceptibility.

The extent to which men and women may differ in susceptibility to DCBs is not known. Available animal data do not provide a clear pattern for gender differences in the toxicity of DCBs, although some subchronic and chronic studies found that males were more sensitive than females for some end points. For example, a multigeneration inhalation study of 1,4-DCB in rats observed increases in adult liver weight that were more pronounced in males than females (Tyl and Neeper-Bradley 1989). In a subchronic oral study of 1,3-DCB in rats, histopathological changes in the thyroid were generally more severe in males than in females (McCauley et al. 1995). This study also found histopathology in the pituitary of male rats, but not female rats. The pituitary lesion was reported to be similar to those induced in gonadectomized rats and was considered to be an indicator of gonadal deficiency (McCauley et al. 1995). Though these animal studies provide an indication that males may be more sensitive to DCBs exposure, the evidence is insufficient for extrapolating to humans.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to dichlorobenzenes. 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 dichlorobenzenes. When specific exposures have occurred, poison control centers and medical toxicologists should be

consulted for medical advice. The following texts provide specific information about treatment following exposures to dichlorobenzenes:

Aaron CK, Howland MA, eds. 1994. Goldfrank's toxicologic emergencies. Norwalk, CT: Appleton and Lange.

Dreisback RH, ed. 1987. Handbook of poisoning. Norwalk, CT: Appleton and Lange.

Ellenhorn MJ, Barceloux, DG, eds. 1997. Medical toxicology: Diagnosis and treatment of human poisoning. New York, NY: Elsevier Publishing.

Grossel TA, Bricker JD. 1994. Principles of clinical toxicology. 3rd edition, New York, NY: Raven Press.

Haddad LM, Winchester JF, eds. 1990. Clinical management of poisoning and drug overdose. 2nd edition, Philadelphia, PA: WB Saunders.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to 1,4-DCB can occur by inhalation, ingestion, or dermal contact. General recommendations for reducing absorption of 1,4-DCB following acute-duration inhalation exposure have included moving the patient to fresh air and administration of 100% humidified supplemental oxygen with assisted ventilation (HSDB 1996). General recommendations for reducing absorption following acute ingestion exposure have included inducing vomiting (unless the patient is or could rapidly become obtunded, comatose, or convulsing, and considering the risk of aspiration of vomitus), gastric lavage, or administration of a charcoal slurry (HSDB 1996). Intake of fatty foods, which would promote absorption, should be avoided. In the case of eye exposure, irrigation with copious amounts of water has been recommended (HSDB 1996). For dermal exposure, and to minimize dermal absorption, the removal of contaminated clothing and a thorough washing of any exposed areas with soap and water has been recommended (HSDB 1996).

3.11.2 Reducing Body Burden

1,4-DCB distributes to fatty tissues and is probably retained there at low concentrations (EPA 1986d; Hawkins et al. 1980; Morita and Ohi 1975; Morita et al. 1975). However, most of an absorbed dose is excreted within 5 days of exposure (Hawkins et al. 1980), and there is no evidence suggesting that the low levels of 1,4-DCB that are likely to remain in fatty tissues would cause adverse effects. For these reasons,

methods for enhancing elimination of 1,4-DCB shortly after high-dose exposure could reduce toxic effects; however, no such methods have been identified. Methods that could enhance the elimination of 1,4-DCB after high- or low-dose exposure in humans or laboratory animals have not been reported.

While it might be possible to develop methods to alter metabolism of 1,4-DCB to promote formation of metabolites that are more easily excreted, this could be difficult because the current lack of knowledge of the specific metabolic pathways of 1,4-DCB precludes speculation concerning which pathways it might be most beneficial to stimulate or inhibit. One pathway for which stimulation may be contraindicated is sulfate conjugate formation (Kimura et al. 1979). Methylation of 1,4-DCB sulfate conjugates can occur, and these methylated conjugates are excreted less rapidly than nonmethylated conjugates (Kimura et al. 1979). Since little is known concerning the toxicity of these conjugates, it is presently not possible to determine the consequences of promoting formation of these metabolites.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The mechanism of action for liver effects of 1,4-DCB has not been clearly delineated; however, based on *in vitro* experiments, induction of P-450 metabolism by pretreatment with phenobarbital may enhance hepatotoxicity (Fisher et al. 1991a). This suggests that one mechanism of hepatotoxicity may be the production of reactive intermediates through phase I P-450-mediated oxidation, although it should be noted that the P-450 inhibitors metyrapone and SKF 525-A did not block hepatotoxicity of 1,4-DCB in human liver tissue *in vitro* (Fisher et al. 1991a). Lattanzi et al. (1989) provide evidence indicating that the microsomal mixed-function oxidase system and microsomal glutathione transferases and, to a lesser degree, cytosolic glutathione transferases, can be involved in the bioactivation of 1,4-DCB. More information concerning the mechanism of action for hepatic effects is needed before methods for blocking that mechanism and reducing toxic effects can be developed.

The mechanisms of action for nephrotoxic (with the exception of $\alpha_{2\mu}$-globulin-mediated nephropathy specific to male rats) or hematotoxic effects have not been clearly delineated, and with the available information, it is difficult to speculate how 1,4-DCB might cause such effects. More information concerning the mechanisms of action for blood and kidney effects are needed before methods for blocking those mechanism and reducing toxic effects can be developed.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of dichlorobenzenes 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 dichlorobenzenes.

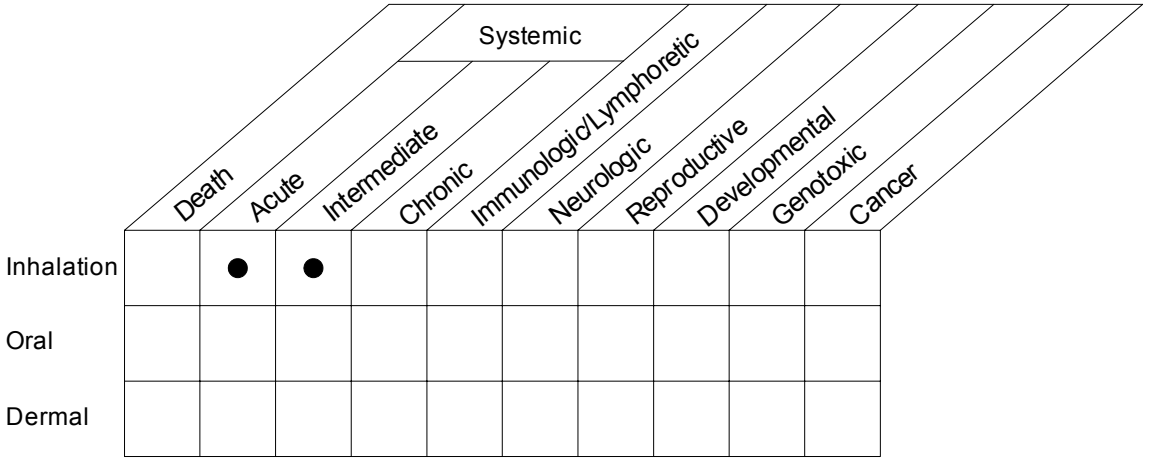
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean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

3.12.1 Existing Information on Health Effects of Dichlorobenzenes

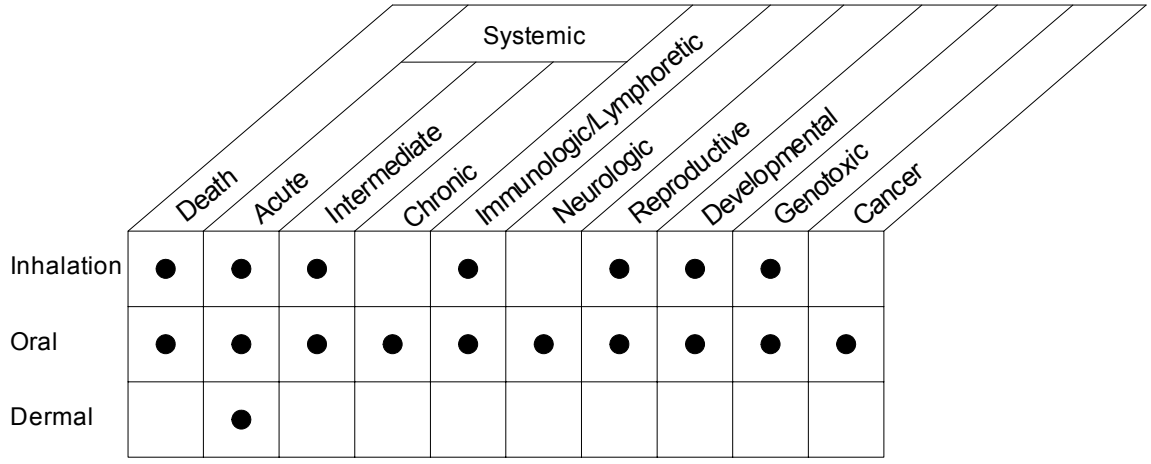
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to dichlorobenzenes are summarized in Figures 3-7, 3-8, and 3-9. The purpose of this figure is to illustrate the existing information concerning the health effects of dichlorobenzenes. Each dot in the figure 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* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Some limited information (i.e., anecdotal, single acute-duration exposure, and workplace exposure) is available on the health effects of human exposure to 1,2- and 1,4-DCB via inhalation and 1,4-DCB by the oral route. For persons exposed via inhalation, there is information on death, systemic effects, neurologic effects. There is also information on systemic effects in humans resulting from acute-, intermediate-, and chronic-duration oral exposure. It is important to note that most of this oral information was obtained from case studies in which levels and durations of exposure to 1,4-DCB were unknown or uncertain.

Figure 3-7. Existing Information on Health Effects of 1,2-Dichlorobenzene



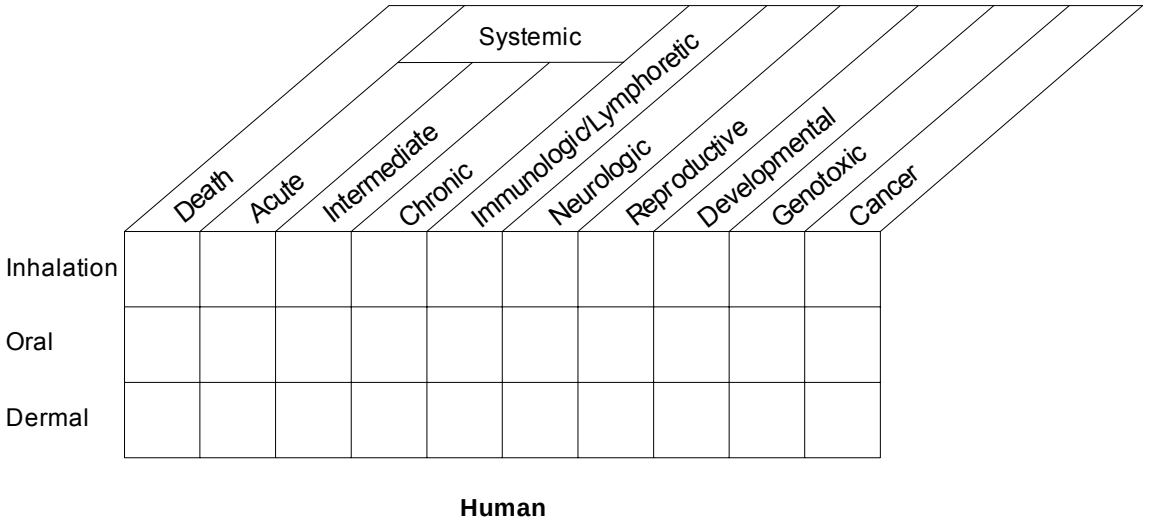
Human



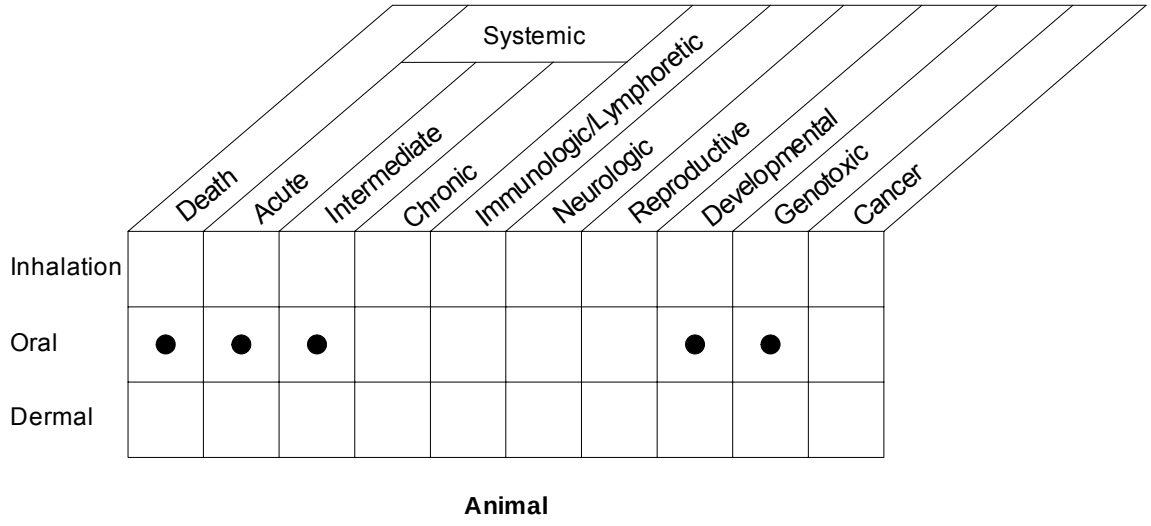
Animal

● Existing Studies

Figure 3-8. Existing Information on Health Effects of 1,3-Dichlorobenzene



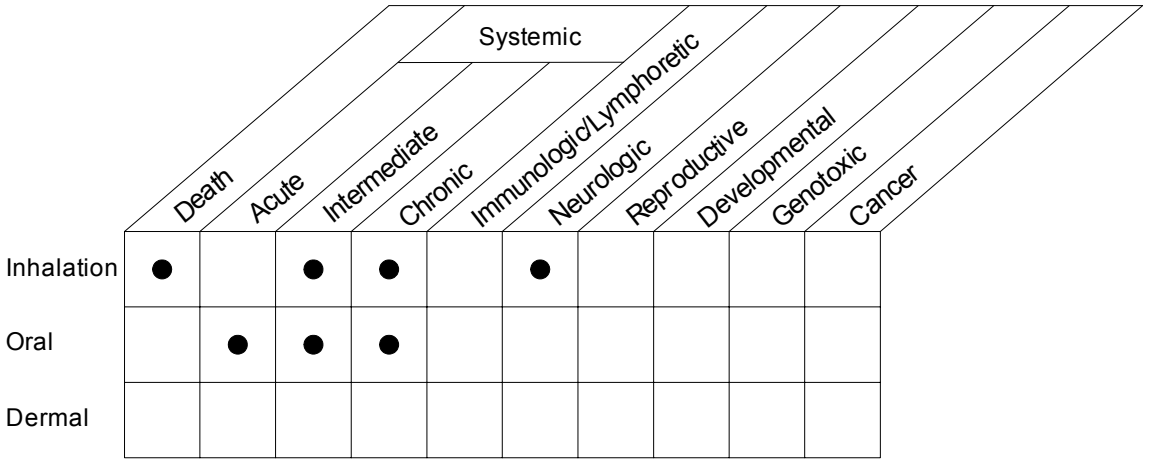
Human



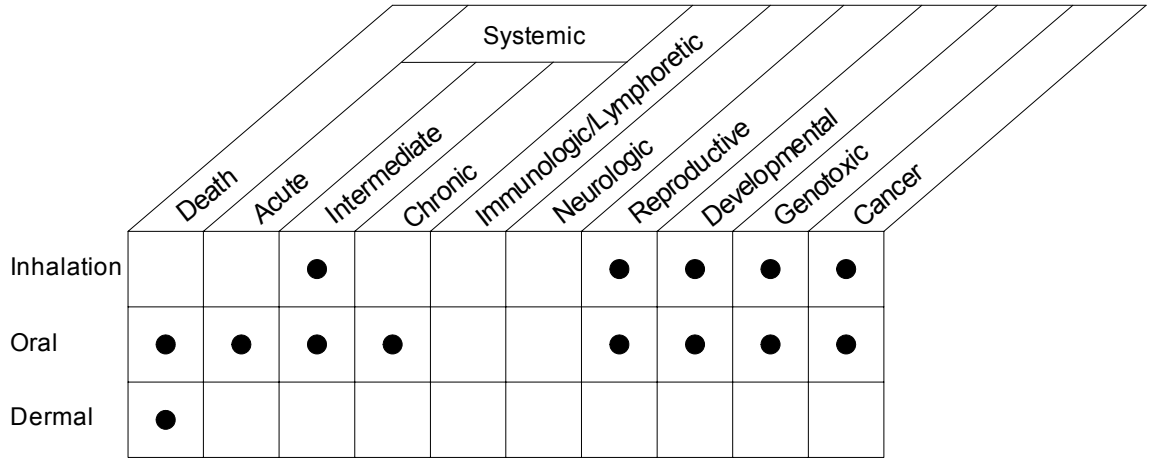
Animal

● Existing Studies

Figure 3-9. Existing Information on Health Effects of 1,4-Dichlorobenzene



Human



Animal

● Existing Studies

Data available on health effects of DCBs in animals are more extensive than in humans. Most of the information is for 1,2- and 1,4-DCB, whereas all data on 1,3-DCB are from one oral study. The most extensively studied isomer is 1,4-DCB. Information is available on the developmental, reproductive, genotoxic, and carcinogenic effects of inhalation exposure to 1,4-DCB, as well as on the systemic effects resulting from intermediate-duration exposure. In studies using oral exposure, information is available on death; systemic effects resulting from acute-, intermediate-, and chronic-duration exposure; and developmental, genotoxic, and carcinogenic effects. Only data on the lack of a lethal effect are available in studies using dermal exposure.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. A limited amount of information is available on health effects in people who were occupationally exposed to 1,2-DCB (Hollingsworth et al. 1958). This information includes exposure levels associated with eye and respiratory tract irritation and results of periodic medical examinations, but the data are insufficient for identifying sensitive systemic end points in humans or for inhalation MRL derivation purposes. The limited information on irritation effects of 1,2-DCB in humans is consistent with histological findings of nasal olfactory epithelial lesions in mice that were intermittently exposed to 1,2-DCB vapor for up to 14 days (Zissu 1995). The severity of the nasal lesions ranged from moderate to severe in severity and occurred at concentrations lower than those that caused acute systemic effects (liver and kidney lesions) in rats (DuPont 1982; Hollingsworth et al. 1958) or developmental effects in rats and rabbits (Hayes et al. 1985). A NOAEL was not identified for the serious nasal effects, precluding derivation of an acute inhalation MRL. Additional studies could characterize the threshold region for nasal effects, confirm that the nasal cavity is more sensitive than systemic end points, and provide a sufficient basis for inhalation MRL derivation.

There is no information on the toxicity of 1,2-DCB in orally-exposed humans. Information on effects of acute oral exposure to 1,2-DCB in animals essentially consists of findings in three systemic toxicity studies in rats and mice (NTP 1985; Rimington and Ziegler 1963; Robinson et al. 1991) and one developmental toxicity study in rats (Ruddick et al. 1983). These studies collectively identify the liver as the most sensitive target, but two are limited by small numbers of animals and lack of a NOAEL due a single dose level (Rimington and Ziegler 1963) or lack of histopathology evaluations at doses lower than the LOAEL (NTP 1985). The third systemic toxicity study (Robinson et al. 1991) is well designed, identified a critical NOAEL and LOAEL for hepatoxicity, and was used to derive an acute oral MRL.

Additional studies are needed to establish whether liver toxicity is the most sensitive end point for acute exposure and the most appropriate basis for the MRL. The oral database for 1,2-DCB particularly lacks adequate assessments of neurotoxicity, immunotoxicity, and end points shown to be sensitive to other DCB isomers (e.g., thyroid and pituitary).

No inhalation toxicity data are available for 1,3-DCB in humans or animals, indicating that a well-designed inhalation toxicity study could provide a basis for an acute inhalation MRL. The acute oral database for 1,3-DCB essentially consists of one well-designed 10-day systemic toxicity study (McCauley et al. 1995) that was sufficient for estimation of an MRL. Additional studies could determine whether the critical effect in this study, increased liver weight, is the most appropriate and sensitive end point for MRL derivation.

A limited amount of information is available on the toxicity of inhaled 1,4-DCB in humans. Case reports of people who inhaled 1,4-DCB provide indications that the liver and nervous system are systemic targets of inhalation toxicity in humans, but are limited by lack of adequate quantitative exposure information and/or verification that 1,4-DCB was the only factor associated with the effects (Cotter 1953; Miyai et al. 1988; Reygagne et al. 1992). An occupational health survey identified odor detection and eye/nose irritation thresholds for 1,4-DCB (Hollingsworth et al. 1956). Information on effects of acute-duration inhalation exposure to 1,4-DCB in animals is available from short-term systemic toxicity studies in rats and guinea pigs (Hollingsworth et al. 1956), a male reproduction study rats (Anderson and Hodge 1976), and developmental toxicity studies in rats and rabbits (Hayes et al. 1985; Hodge et al. 1977). These animal studies identified the lung as the target of concern, and are consistent with chronic inhalation data (Aiso et al. 2005b; Japan Bioassay Research Center 1995) as well as the human occupational experience (Hollingsworth et al. 1956), but are insufficient for deriving an acute inhalation MRL. Studies in animals investigating potentially sensitive systemic end points (e.g., respiratory, endocrine, neurological, immunological) are needed to identify an appropriate end point and effect level for MRL derivation.

Information on effects of non-lethal acute-duration oral exposures to 1,4-DCB is essentially limited to hepatic and renal changes of unclear toxicological significance observed in animal studies designed to elucidate mechanisms of liver and kidney toxicity in rats and mice. Appropriately designed acute oral studies are needed to provide a suitable basis for MRL derivation.

The only available study using the dermal route is a lethality study that attempted to determine a dermal LD_{50} level for 1,4-DCB in rats (Gaines and Linder 1986). There are no available toxicokinetic data that

have examined absorption of 1,4-DCB via the dermal route. If dermal absorption and systemic distribution of 1,4-DCB could be demonstrated, acute-duration studies using this route would be useful since humans are commonly exposed to it by handling various consumer products in the home and being exposed to the vapor form.

Intermediate-Duration Exposure. Information on the toxicity of intermediate-duration inhalation exposure to 1,2-DCB is limited to the findings of a multispecies subchronic study (Hollingsworth et al. 1958) and a 2-generation reproduction study in rats (Bio/dynamics 1989). These studies identified NOAELs and LOAELs for liver and body weight effects, but possible effects in the nasal cavity, a known sensitive target of 1,2-DCB based on acute data, were not evaluated. Derivation of an intermediate-duration inhalation MRL for 1,2-DCB is precluded because the acute-duration serious LOAEL for nasal effects (Zissu 1995) is lower than the available intermediate-duration LOAELs for systemic and developmental effects. Additional studies could verify the nasal cavity is more sensitive than systemic end points and provide exposure-response data useful for inhalation MRL derivation.

No information was located regarding the toxicity of inhaled 1,3-DCB in humans or animals, indicating that appropriate studies are needed to provide a basis for derivation of an intermediate-duration inhalation MRL for this isomer. The database for intermediate-duration oral exposure to 1,3-DCB consists of one well-designed 90-day systemic toxicity study (McCauley et al. 1995) that was sufficient for estimation of an intermediate oral MRL. The thyroid, pituitary, and liver were identified as sensitive targets and incidences of pituitary lesions were used to derive an intermediate oral MRL.

Case studies are available on humans exposed to 1,4-DCB via inhalation and the oral route for intermediate-duration exposure. These include the report of a 69-year-old man who developed skin discolorations and swelling of his hands and feet after about 3 weeks of exposure to 1,4-DCB in his home (Nalbandian and Pearce 1965), the cases of a 60-year-old man and his wife who both died of liver atrophy after their home had been saturated with moth ball vapor for 3–4 months (Cotter 1953), and the case of a 21-year-old woman who developed hypochromic, microcytic anemia as a result of ingesting 1,4-DCB toilet air freshener blocks throughout pregnancy (Campbell and Davidson 1970). All of these case studies lack critical dosing amounts and durations. It would be helpful if future reports of accidental or intentional exposure included dose information (measured or estimated) that could be used to help characterize dose-response relationships in humans.

Information on effects of intermediate-duration inhalation exposure to 1,4-DCB in animals is available from a multispecies subchronic toxicity study (Hollingsworth et al. 1956), a 13-week toxicity study in rats and mice (Aiso et al. 2005a), and a 2-generation reproductive/developmental toxicity study in rats (Tyl and Neeper-Bradley 1989). The 13-week and 2-generation studies identified a NOAEL and LOAEL for increased relative liver weight, and increased liver weight was used to derive an MRL. A chronic inhalation study (Aiso et al. 2005b; Japan Bioassay Research Center 1995) found that nasal lesions in rats and testicular effects in mice were more sensitive than liver effects. No nasal or testicular lesions were reported in the 13-week rat and mouse study, and these tissues were not examined in the multispecies subchronic study. Additional studies could verify that liver weight is the most appropriate basis for the intermediate inhalation.

Information on the systemic toxicity of intermediate-duration oral exposure to 1,4-DCB is available from a number of studies conducted in rodents, mainly rats and mice, as well as one study in dogs (Bomhard et al. 1988; Hollingsworth et al. 1956; NTP 1985; Lake et al. 1997; Naylor and Stout 1996; Umemura et al. 1998). Liver and kidney effects were the most consistently observed, best characterized, and most sensitive findings in these studies. Liver effects were used as the basis for intermediate-duration oral MRLs for 1,2-DCB (NTP 1985) and 1,4-DCB (Naylor and Stout 1996).

Studies using the dermal route for intermediate-duration exposure would be useful if absorption and systemic distribution of 1,4-DCB by this route could first be demonstrated in toxicokinetic studies.

Chronic-Duration Exposure and Cancer. No studies were located regarding the chronic inhalation toxicity of 1,2-DCB in humans or animals, indicating that data are needed to provide a basis for estimation of an inhalation MRL. Regarding chronic oral toxicity of 1,2-DCB, the only available study is a two-dose-level NTP (1985) bioassay that was conducted in rats and mice. The only exposure-related effect in either species was a significantly increased incidence of renal tubular regeneration in male mice. A NOAEL and LOAEL were identified for this lesion and incidences of renal tubular regeneration were used to derive a chronic oral MRL for 1,2-DCB. No information is available on the carcinogenicity of 1,2-DCB in humans. Data on cancer in animals are limited to the NTP (1985) chronic bioassay, in which no exposure-related tumors were found in male and female rats and mice exposed to two dose levels of 1,2-DCB for 103 weeks. This is a well-designed chronic study with respect to exposure duration and scope of histological examinations, but it is uncertain whether an MTD was achieved in either species. Additional studies that include multiple dose levels and clear MTDs, as well as toxicity end points that could be more sensitive than kidney lesions (e.g., endocrine and immunological), could be used determine

if the MRL is based on the most appropriate effect level and also provide an better assessment of carcinogenic potential.

No studies were located regarding the chronic inhalation or oral toxicity of 1,3-DCB in humans or animals, indicating that data are needed to provide the bases for chronic MRL and carcinogenicity assessments.

Several case studies of chronic human exposure to 1,4-DCB have been reported. Reported effects resulting mainly from chronic inhalation included pulmonary granulomatosis in a 53-year-old woman who had been inhaling 1,4-DCB crystals in her home for 12–15 years (Weller and Crellin 1953); atrophy and cirrhosis of the liver in a 34-year-old woman who was exposed to 1,4-DCB-containing products in a small enclosed booth in a department store for 1 or more years (Cotter 1953); jaundice and liver atrophy in a 52-year-old man after 2 years of exposure to 1,4-DCB in the fur storage plant where he worked (Cotter 1953); and ataxia, speech difficulties, limb weakness, and altered brainwave activity in a 25-year-old woman who had been exposed to high concentrations of 1,4-DCB in her bedroom, bedding, and clothes for about 6 years (Miyai et al. 1988). A limited occupational health survey reported that nasal and ocular irritation, but no major systemic health effects, were the only 1,4-DCB-related complaints (Hollingsworth et al. 1956). Further occupational health data on individuals exposed chronically to 1,4-DCB would be useful for both cancer and noncancer health effect end points already mentioned. The only data located relating to chronic oral human exposure to 1,4-DCB come from a case report of a 19-year-old black woman who developed an increase in skin pigmentation as a result of eating 1,4-DCB moth pellets daily for about 2.5 years (Frank and Cohen 1961). All of these case studies lacked dosing amounts and durations, which makes it difficult to characterize dose-response relationships for effects in humans exposed to 1,4-DCB. No studies of chronic dermal exposure to 1,4-DCB were located, although it seems likely that chronic inhalation and oral exposure scenarios, both in the home and in the workplace, have also involved dermal contact with 1,4-DCB.

A limited amount of additional information is available on the chronic toxicity of inhaled 1,4-DCB in humans. Periodic health examinations of workers who were exposed to 1,4-DCB for an average of 4.75 years (range, 8 months to 25 years) showed no changes in standard blood and urine indices (Hollingsworth et al. 1956). The data from this occupational study are inadequate for chronic MRL derivation due to poor characterization of exposure levels, insufficient investigation of systemic health end points, and poor reporting as well as other study deficiencies. However, eye and nose irritation findings in this study are consistent with nasal effects observed in chronically exposed animals.

Information on the chronic inhalation toxicity of 1,4-DCB in animals is available from two studies in rats and mice (Aiso et al. 2005b; Japan Bioassay Research Center 1995; Riley et al. 1980a, 1980b). One of these studies (Aiso et al. 2005b; Japan Bioassay Research Center 1995) identified nasal lesions in rats and provided a sufficient basis for MRL estimation.

Information on the chronic oral effects of 1,4-DCB is available from one study each in rats, mice, and rabbits (Hollingsworth et al. 1956; NTP 1987). Lesions were observed in the kidneys and liver, and the lowest tested dose was a LOAEL for renal effects in rats (NTP 1987). Naylor and Stout (1996) identified liver effects (increased liver weight, changes in liver enzymes, and histopathology) in dogs administered 1,4-DCB for 1 year; these liver effects provided a sufficient basis for chronic oral MRL estimation. Information on carcinogenicity of 1,4-DCB is available from the chronic oral and inhalation studies in rats and mice. The oral study (NTP 1987) found evidence of carcinogenicity based on increased tumor incidences in male rat kidneys and in the livers of male and female mice. The kidney tumors are not relevant to humans because the mechanism ($\alpha_{2\mu}$-globulin nephropathy) is specific to male rats. One of the inhalation studies (Aiso et al. 2005b; Japan Bioassay Research Center 1995) similarly showed tumor induction in the livers of male and female mice, although there was no tumor formation in either sex of rats. The other inhalation study (Riley et al. 1980a, 1980b) found no neoplastic changes in rats or mice, but the adequacy of the study for carcinogenicity evaluation is limited by failure to reach the maximum tolerated dose, less-than-lifetime exposure durations, and short observation periods in both species. There is sufficient evidence of 1,4-DCB carcinogenicity in animals based on the induction of liver tumors in mice exposed by both the oral and inhalation routes. Unlike the kidney tumors in male rats, the mechanistic basis of the liver tumors in mice is not adequately defined, indicating that additional studies could help to better assess their relevance to humans.

Data on the effects of chronic dermal exposure to 1,4-DCB might be useful if dermal absorption and systemic distribution of 1,4-DCB can be demonstrated from toxicokinetic studies, since chronic dermal exposure to 1,4-DCB occurs as a result of bathing and showering in drinking water that contains low levels of this chemical in many U.S. communities.

Genotoxicity. Genotoxic effects of 1,2- and 1,3-DCB have been investigated in various animal test systems with generally mixed results. The genotoxicity of 1,4-DCB has been extensively studied in a wide variety of *in vitro* and *in vivo* animal assays with a preponderance of negative results. Additional studies could help to clarify the mechanism of carcinogenesis for 1,4-DCB-induced liver tumors in mice.

There are considerable data supporting a sustained proliferative response following 1,4-DCB exposure as the mode of action for liver tumor formation; however, the existing evidence is incomplete.

Reproductive Toxicity. The reproductive toxicity of 1,2-DCB has been evaluated in a 2-generation inhalation study in rats (Bio/dynamics 1989), but not by the oral route. The inhalation study found no effects on reproduction in either generation at exposure levels higher than those causing liver effects in the parental animals, indicating that it can be used to partially address the data gap for oral exposure.

No information was located on possible reproductive effects of 1,3-DCB, indicating that reproductive toxicity is a data need for both inhalation and oral exposure to this isomer.

The reproductive toxicity of 1,4-DCB has been evaluated in inhalation and oral 2-generation studies in rats with no exposure-related effects on reproductive function (Bornatowicz et al. 1994; Tyl and Neeper-Bradley 1989). An inhalation study of male mice exposed to 1,4-DCB for 5 days did not find an adverse impact on their ability to impregnate females (Anderson and Hodge 1976). Incidences of morphologically abnormal sperm were increased in rats that were intraperitoneally injected with 1,4-DCB (Murthy et al. 1987). Histopathology evaluations of 1,4-DCB-exposed animals have not demonstrated changes in reproductive tissues in the preponderance of studies. Based on the available data, there is no compelling need for additional reproductive toxicity studies of 1,4-DCB.

Developmental Toxicity. The developmental toxicity of inhaled 1,2-DCB was evaluated in an adequate study of gestationally-exposed rats and rabbits (Hayes et al. 1985). Skeletal variations, but no teratogenic effects, occurred in rats at a concentration that also caused maternal toxicity. A poorly reported oral study in which rats were gestationally exposed to 1,2-DCB (Ruddick et al. 1983) found no effects on fetuses and indicates that developmental toxicity, if induced, would only occur at levels that were maternally toxic. No information is available on possible neurodevelopmental effects of 1,2-DCB, indicating that this is a data need.

No information was located on the developmental toxicity of 1,3-DCB, indicating that this is a data need for both inhalation and oral exposure to this isomer.

The developmental toxicity of inhaled 1,4-DCB was evaluated in adequate studies of gestationally-exposed rats and rabbits (Hayes et al. 1985; Hodge et al. 1977). No maternal or prenatal developmental toxicity occurred in the rats, although there was evidence of fetotoxicity (a minor variation of the

circulatory system) in the rabbits at a concentration that was maternally toxic and higher than LOAELs for systemic toxicity in other studies. Information on developmental toxicity of ingested 1,4-DCB is available from an 2-generation oral study in rats (Bornatowicz et al. 1994). Fetuses were not examined for prenatal changes, but various effects occurred in the offspring perinatally and during the later pre-weaning period, including decreased neonatal survival and impaired neurobehavioral development in F_1 and F_2 pups. This finding suggests that postnatal neurobehavioral development is a sensitive end point for 1,4-DCB that could be better characterized by additional studies.

Immunotoxicity. No information is available on immunological function in humans or animals exposed to 1,2-DCB or 1,3-DCB by the inhalation or oral routes. Lymphoid depletion in the thymus was observed histologically in rats that were exposed to a high oral dose of 1,2-DCB for 13 weeks (NTP 1985), suggesting that the immune system is a possible target of concern and providing an additional indication of the need for adequate assessments of immunotoxicity.

No studies were located that directly assess the potential immunotoxic effects of 1,4-DCB in humans exposed by inhalation, oral, or dermal routes. However, case reports of skin reactions in a 69-year-old man who was exposed via inhalation (Nalbandian and Pearce 1965) and a 19-year-old woman who ingested moth pellets (Frank and Cohen 1961) suggest that the immune system may be a target for 1,4-DCB. Oral exposure to high doses of 1,4-DCB for 13 weeks caused lymphoid necrosis in the thymus, lymphoid depletion in the spleen, and hematopoietic hypoplasia in the spleen and bone marrow of mice, and lymphoid depletion of the thymus and spleen in rats (NTP 1987). Effects of oral 1,4-DCB exposure on function of the immune system have not been studied, although there were no functional decrements in a 12-week inhalation immunotoxicity study in guinea pigs that assessed a limited number of indices (Suzuki et al. 1991). Comprehensive immunological testing would help to adequately assess the immunotoxic potential of 1,4-DCB.

Neurotoxicity. Comprehensive neurobehavioral assessments have not been performed for any of the DCB isomers. Clinical signs neurotoxicity (e.g., ataxia and clonic contractions) were observed in rats that were orally exposed to a high dose of 1,2-DCB for 15 days (Rimington and Ziegler 1963), but similar effects were not found in rats or mice in other studies of this isomer. No signs of neurotoxicity occurred in rats were orally exposed to 1,3-DCB for up to 90 days (McCauley et al. 1995).

Neurological effects including dizziness, weakness, headaches, nausea, vomiting, numbness, clumsiness, speech difficulties, and altered patterns of certain brainwaves have been reported to have occurred in case

studies of persons exposed to 1,4-DCB via inhalation (Cotter 1953; Miyai et al. 1988), as well as with other halogenated hydrocarbons. There are no data on neurological effects in humans exposed to 1,4-DCB through the oral or dermal routes. Neurotoxic effects of 1,4-DCB occurred in rats, rabbits, and guinea pigs following inhalation exposure to high concentrations; effects included tremors, weakness, and periods of unconsciousness. Similar neurological responses were observed following oral exposure to high doses of 1,4-DCB (NTP 1987; Rimington and Ziegler 1963). No studies were located that reported neurological effects after a dermal route of exposure. Additional information, particularly on subtle behavioral changes at low levels of inhalation and oral exposure, is needed to adequately assess the neurotoxic potential of 1,4-DCB and for quantifying dose-response relationships.

Epidemiological and Human Dosimetry Studies. A limited amount of information is available on the inhalation toxicity of 1,2- and 1,4-DCB in humans from observations in exposed workers, mainly from assessments of symptoms and standard blood and urine indices as determined by periodic occupational health examinations (Hollingsworth et al. 1956, 1958). No information is available on the toxicity of ingested 1,2- or 1,3-DCB in humans. Information on toxic effects of 1,4-DCB in orally exposed humans is limited to two case reports describing hematological changes, particularly anemia, following known or presumed repeated ingestion of unknown doses of the compound in commercial products (Campbell and Davidson 1970; Hallowell 1959). The limited available information suggests that inhalation or oral exposure to DCBs can cause effects in humans similar to those found in animals, particularly in the respiratory tract, liver, and hematological systems. There are no case studies or epidemiological data that suggest that levels of DCBs found in the environment are associated with significant human exposure. The available data suggest that levels of DCBs in outside air are relatively insignificant, although the compounds are widespread (IARC 1982; Scuderi 1986; Wallace et al. 1986b). Levels in groundwater and surface water are also relatively low (Coniglio et al. 1980; Dressman et al. 1977; IJC 1989; Oliver and Nicol 1982a; Page 1981; Staples et al. 1985). These observations indicate that the most likely population to exhibit effects of DCB exposures would be occupationally exposed groups. Human epidemiological studies that provide a more definitive dose-response relationship between exposure, clinical manifestations, and target organ toxicity (i.e., hepatic, hematological, and neurological systems) would be useful.

Biomarkers of Exposure and Effect.

Exposure. Exposure to DCBs can be identified by measuring levels of the isomers in blood (Bristol et al. 1982; Hill et al. 1995; Jan 1983; Langhorst and Nestrick 1979; Pellizzari et al. 1985), urine (Ghittori et al.

1985; Hill et al. 1995; Kumagai and Matsunaga 1995, 1997; Zenser et al. 1997), adipose tissue (Jan 1983), and breast milk (Jan 1983; Mes et al. 1986), as well as metabolites in the urine. Urinary 2,5-dichlorophenol is a well-documented biomarker for monitoring worker exposure to 1,4-DCB (McKinney et al. 1970; Pagnotto and Walkley 1965), and urinary 2,3- and 3,4-dichlorophenols, as well as 3,4- and 4,5-dichlorocatechols, have been shown to be useful indicators of exposure to 1,2-DCB (Kumagai and Matsunaga 1997). Additional data with which to correlate these measurements to exposure levels, particularly by the inhalation route, and potential health effects, would be useful.

Effect. There are no health effects that are uniquely associated with exposure to DCBs. Therefore, studies to identify a specific biomarker of effect for DCBs would be useful.

Absorption, Distribution, Metabolism, and Excretion. There are no data on the toxicokinetics of any DCB isomer in humans. Experiments with laboratory animals indicate that DCBs are absorbed via oral or inhalation exposure and distributed mainly to adipose tissue, with some distribution to the liver and kidney, and minor amounts to other organs (Hawkins et al. 1980; Kimura et al. 1979). Absorbed DCBs are principally metabolized to dichlorophenol metabolites (e.g., 2,5-dichlorophenol from 1,4-DCB) by oxidation and is rapidly eliminated, primarily in urine (Azouz et al. 1955; Hawkins et al. 1980). The available data indicate that the route of exposure is likely to have little effect on the subsequent metabolism and excretion of DCBs. Scant data are available on absorption and systemic distribution resulting from exposure via the dermal route. Dermal absorption data would be particularly useful considering that the inhalation MRLs are based on whole-body exposure. 1,4-DCB produces a burning sensation when applied to the skin for a prolonged period of time, indicating at least minimal penetration to nerve endings within the skin (Hollingsworth et al. 1956). The little information that is available suggests that dermal exposure is associated with low systemic toxicity in both humans and laboratory animals. It would be useful to confirm this because it could provide a basis for assessing the likelihood of toxic effects resulting from dermal exposure and the need to conduct various toxicity studies via the dermal route. Additional toxicokinetic data would be useful for quantitating route-specific absorption rates.

Comparative Toxicokinetics. There are no available studies that compare the toxicokinetics of any of the DCB isomers across species. This has been an important area of concern in interpreting the results of animal studies with 1,4-DCB with respect to their relevance to humans, most notably in the observations of renal toxicity and carcinogenicity in male rats. Although this specific issue has been largely resolved, it would be useful to have further data comparing the toxicokinetics of 1,4-DCB across

species in order to understand better which animal model is likely to compare most directly with humans with regard to other toxic effects in response to 1,4-DCB exposure. From the available data in humans and laboratory animals, the primary metabolite produced after exposure to 1,4-DCB is 2,5-dichlorophenol. This metabolite appears mainly in the urine after undergoing phase II metabolism, principally to the sulfate and glucuronide conjugates, with some exiting via the bile (Azouz et al. 1955; Fisher et al. 1995; Hissink et al. 1997a; Hallowell 1959; Kimura et al. 1979; Klos and Dekant 1994).

Methods for Reducing Toxic Effects. Based on the chemical and physical properties of DCBs, absorption is most likely to occur by passive diffusion. However, this has not been investigated. Studies that investigate the mechanism by which DCBs are absorbed could be useful in developing methods for reducing its absorption. Standard methods exist for reducing the absorption of DCBs across the skin, lungs, and gastrointestinal tract (HSDB 1996) and are described in more detail in Chapter 7 of this profile; however, none of these are specific for exposures to 1,2-, 1,3-, or 1,4-DCB. DCBs can be retained in fatty tissues at low levels (EPA 1986f; Hawkins et al. 1980; Morita and Ohi 1975; Morita et al. 1975). Additional studies that characterize the metabolic pathways that enhance excretion may be useful in developing a method for reducing body burden. However, since most of an absorbed dose is likely to be eliminated within several days (Hawkins et al. 1980), it seems unlikely that methods for reducing body burden would be of much benefit. There is limited evidence that DCBs are metabolically activated to hepatotoxic intermediates (Fisher et al. 1991a; Lattanzi et al. 1989). Additional studies that further characterize the metabolic activation of DCBs could be useful for understanding how metabolites interact and to develop methods for interfering with the mechanism of action.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

Essentially all of the studies on effects of exposure of humans to DCBs have focused on adults. It is unknown whether children differ from adults in their susceptibility to health effects from DCBs. Only two case reports of 1,4-DCB specifically referenced potential exposure to a child (Campbell and Davidson 1970; Hallowell 1959). Data relating to health effects in general for children are lacking. There are no data describing the developmental effects in humans. Such data, although potentially useful, would be difficult to obtain. See the Developmental Toxicity subsection above for related data needs.

Although there is no reason to suspect that the pharmacokinetics of DCBs differs in children and adults, scant data are available to support or disprove this statement. Studies of absorption, distribution, metabolism, and excretion in children would aid in determining if children are at an increased risk, particularly if conducted in an area where a high-dose acute or low-dose chronic exposure to an environmental source were to occur. With regard to exposure during development, additional research on maternal and fetal/neonatal toxicokinetics, placental biotransformation, the mechanism of action in children, and the risk associated with the transfer of DCBs to an infant via breast milk would be useful in obtaining a more complete picture of prenatal and neonatal development. Direct evidence on whether DCBs crosses the placenta and on the kinetics associated with that transfer is also needed. Data needs exist for determining if specific biomarkers of exposure or effect exist in children (and how those differ from adults) and how DCBs interact with other chemicals (i.e., other organochlorine pesticides, drugs, etc.) Data needs also exist for methods to reduce peak absorption after exposure, to reduce body burden, and to interfere with the mechanism of action for toxic effects targeted for adults as well as for children.

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

3.12.3 Ongoing Studies

No known ongoing studies related to the toxicity or toxicokinetics of DCBs were identified.