2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of 4,4´-methylenedianiline. 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.

Methylenedianilines can exist in five isomeric forms: 2,2'-methylenedianiline; 2,4'-methylenedianiline; 3,3'-methylenedianiline; 3,4'-methylenedianiline; and 4,4´-methylenedianiline. Of the various isomers, 2,2'-methylenedianiline, 3,4'-methylenedianiline, 3,3'-methylenedianiline, and 2,4'-methylenedianiline are produced on a very small scale as a research chemical (HSDB 1996). The isomer 4,4´-methylenedianiline is produced in the United States for industrial use. Therefore, this profile will limit its discussion to 4,4´-methylenedianiline.

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

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure-inhalation, oral, and dermal; and then by health effect-death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (15-364 days), am 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 (LOAEL) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of 4,4′-methylenedianiline are indicated in Tables 2-2 and 2-3 and Figure 2-2.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for 4,4′-methylenedianiline. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

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

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

2.2.1 Inhalation Exposure

Occupational exposure to 4,4´-methylenedianiline probably involves both inhalation of dust particles containing the chemical, and dermal contact with these particles. In addition, direct ingestion of contaminated dust or ingestion of particles that are expelled from the respiratory tree cannot be ruled out. It is generally agreed, however, that dermal contact is the main contributing route of exposure in occupational settings. For this reason, health effects in humans attributed to occupational exposure to 4,4´-methylenedianiline are discussed in Section 2.2.3, Dermal Exposure.

2.2.1.1 Death

No studies were located regarding death in humans or animals after inhalation exposure to 4,4´-methylenedianiline.

2.2.1.2 Systemic Effects

No studies were located regarding systemic effects in humans after inhalation exposure to 4,4´-methylenedianiline. In addition, no studies were located regarding cardiovascular, gastrointestinal, hematological, or musculoskeletal effects in animals after inhalation exposure to 4,4´-methylenedianiline. Only one study was located that provided some information on systemic effects of inhaled 4,4´-methylenedianiline in animals; this limited information is summarized below.

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

Respiratory Effects. Guinea pigs exposed nose-only to 440 mg/m^3 of an aerosol of 4,4′-methylenedianiline in propylene glycol 4 hours per day, 5 days per week for 2 weeks experienced no respiratory distress during exposures (Leong et al. 1987). The mean particle diameter was 2.4 µm. Two weeks after exposures terminated and prior to sacrifice, tests were conducted to detect possible changes in the distensibility of the lungs from an intratracheal challenge dose of 4,4′-methylenedianiline; the results were unremarkable. Histopathologic examination of the lungs revealed mild to slight pneumonia and pulmonary granulomas.

Hepatic Effects. No histopathological alterations were observed in the liver of guinea pigs exposed nose-only to 440 mg/m^3 of an aerosol of 4,4′-methylenedianiline 4 hours per day, 5 days per week for 2 weeks (Leong et al. 1987). The animals were sacrificed 2 weeks after the exposure period terminated. No further information regarding hepatic effects after inhalation exposure to 4,4′-methylenedianiline was located.

Renal Effects. No histopathological alterations were observed in the kidneys of guinea pigs exposed nose-only to 440 mg/m^3 of an aerosol of 4,4′-methylenedianiline 4 hours per day, 5 days per week for 2 weeks (Leong et al. 1987). Sacrifices were conducted 2 weeks after the exposure period terminated. No further information regarding renal effects following inhalation exposure to 4,4′-methylenedianiline was located.

Dermal Effects. The possibility that inhalation exposure to 4,4′-methylenedianiline could induce dermal sensitization was explored in guinea pigs (Leong et al. 1987) (see Section 2.2.3.2). The animals were exposed nose-only to 440 mg/m^3 of an aerosol of 4,4′-methylenedianiline 4 hours per day, 5 days per week for 2 weeks. Two weeks after exposure ceased, 4,4′-methylenedianiline (0.2-22 mg/kg) was applied to shaved sites of skin and observations were made for up CO 24 hours. Neither erythema nor edema were observed suggesting that under the conditions of the experiment, 4,4′-methylenedianiline was not a dermal sensitizer. No further information was located regarding dermal effects after inhalation exposure to 4,4′-methylenedianiline.

Table 2-1. Levels of Significant Exposure to 4,4'-Methylenedianiline - Inhalation

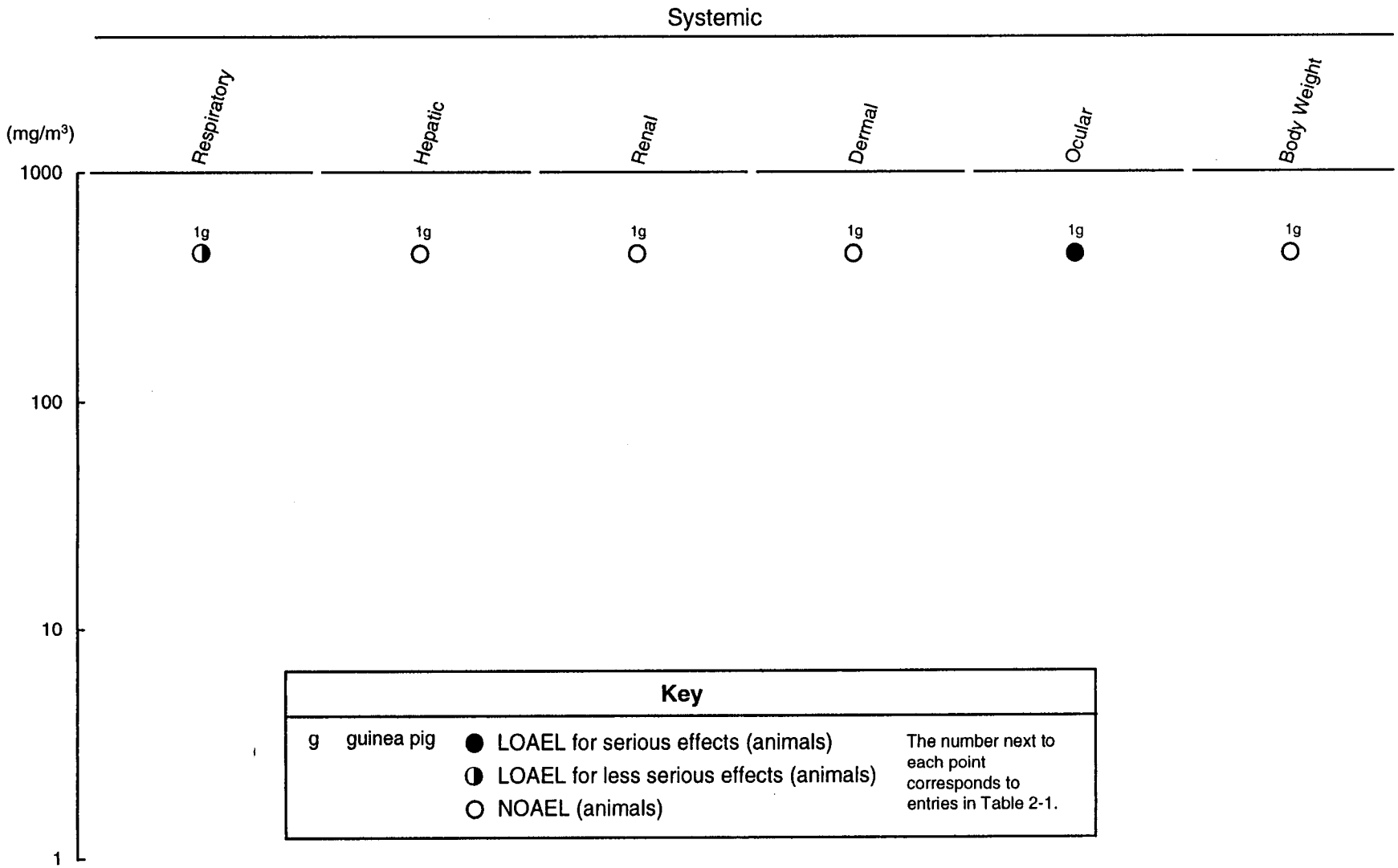
Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL		Reference
					Less serious (mg/m3)	Serious (mg/m3)	
ACUTE EXPOSURE							
Systemic							
1	Gn Pig (Albino Hartley)	2 wk 5 d/wk 4 hr/d	Resp		440M (mild to slight pneumonia in 3/16 animals; pulmonary granulomas in 7/16)		Leong et al. 1987
			Hepatic	440 M			
			Renal	440 M			
			Dermal	440 M			
			Ocular			440 M (degeneration of photoreceptor cells in retina)	
			Bd Wt	440 M			

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

Bd Wt = Body Weight; d = day(s); Gn Pig = guinea pig; hr = hour; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Figure 2-1. Levels of Significant Exposure to 4,4-Methylenedianiline - Inhalation

Acute (≤14 days)



Ocular Effects. The role of melanin in ocular toxicity of 4,4′-methylenedianiline was examined in guinea pigs (Leong et al. 1987). Two strains were used, albino (lacking melanin) and pigmented. The animals were exposed nose-only to 440 mg/m^3 of an aerosol of 4,4′-methylenedianiline 4 hours per day, 5 days per week for 2 weeks. Two weeks after exposure terminated, the animals were sacrificed and the retinas examined. The retinas of both strains showed marked alterations ranging from retraction and thickening of the outer segments of the photoreceptor cells to swelling and retraction extended through the inner segments of the photoreceptors to the outer nuclear layer. There were also degenerative changes in the pigmented epithelial cells. Since the retinal lesions were similar in both strains, the authors concluded that these changes were not related to the affinity of 4,4′-methylenedianiline for melanin. No further information was located regarding ocular effects after inhalation exposure to 4,4′-methylenedianiline.

Body Weight Effects. Guinea pigs exposed nose-only to 440 mg/m^3 of an aerosol of 4,4′-methylenedianiline 4 hours per day, 5 days per week for 2 weeks experienced a slight weight loss during exposure days, but recovery was apparent during the 2 resting weekend days (Leong et al. 1987). The authors attributed this temporary loss in weight to the stress of being restrained during exposure since the trend over the entire experimental period was similar between exposed and control animals. No further information was located regarding body weight effects after inhalation exposure to 4,4′-methylenedianiline.

No studies were located regarding the following health effects in humans or animals after inhalation exposure to 4,4′-methylenedianiline:

2.2.1.3 Immunological and Lymphoreticular Effects
2.2.1.4 Neurological Effects
2.2.1.5 Reproductive Effects
2.2.1.6 Developmental Effects
2.2.1.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

No studies were located regarding carcinogenic effects in humans or animals after inhalation exposure to 4,4′-methylenedianiline.

2.2.2 Oral Exposure

2.2.2.1 Death

No cases of human deaths attributed to oral exposure to 4,4′-methylenedianiline were located in the literature reviewed. The only relevant information was found in a study that examined the potential long-term health effects in a population that had consumed bread contaminated with 4,4′-methylenedianiline in 1965 in the Epping district of Essex, England (Hall et al. 1992). Liver toxicity was the main adverse effect reported at the time of the accident (Kopelman et al. 1966). Of the original 84 cases, 55 people were alive, 18 had died, and 16 could not be traced (Hall et al. 1992). The causes of death (neoplastic and non-neoplastic diseases) were, by and large, unremarkable, and the observed/expected ratios for death from all causes were well below 1.0. Thus, there was no obvious relationship between ingestion of 4,4′-methylenedianiline in that particular episode and death in humans.

Several studies have reported death in animals after oral administration of 4,4′-methylenedianiline. In Wistar rats, oral LD_{50} values of 335 mg/kg (Schmidt et al. 1980) and 830 mg/kg (Pludro et al. 1969) were reported. In the former study, the test material was administered in propylene glycol, whereas peanut oil was used as vehicle in the latter. Two of 5 male mice died in a 14-day study after receiving daily doses of 207 mg 4,4′-methylenedianiline/kg/day in the drinking water, 1 of 5 females died at a 220 mg/kg/day dose level; all males and females (5/5) died at 829 mg/kg/day and 882 mg/kg/day, respectively (NTP 1983). The cause of death was not reported. The LD_{50} values in male guinea pigs and male rabbits administered 4,4′-methylenedianiline in peanut oil were 260 mg/kg and 620 mg/kg, respectively (Schmidt et al. 1974). In an intermediate-duration study, female Sprague-Dawley rats were treated by gavage intermittently for 30 days with 36 mg 4,4′-methylenedianiline/kg/day in sesame oil (Griswold et al. 1968). Forty-five days after treatment started, survival in treated rats was reduced 16% relative to untreated controls; the cause of death was not reported. In a chronic-duration study, survival rate was reduced by approximately 20% in male $B6C3F_1$ mice

administered doses of 57 mg 4,4′-methylenedianiline/kg/day in the drinking water for 103 weeks (Lamb et al. 1986; NTP 1983).

LOAEL and LD_{50} values for death for each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

Little information is available regarding systemic effects in humans after oral exposure to 4,4′-methylenedianiline. In contrast, numerous studies have examined the effects of oral administration of 4,4′-methylenedianiline in animals, particularly in rats. The overall evidence suggests that the liver and perhaps the thyroid are target organs for 4,4′-methylenedianiline toxicity.

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

Respiratory Effects. Abnormal respiratory rhythm was reported in a man on arrival to the hospital after drinking an unspecified amount of a liquid containing 4,4′-methylenedianiline, potassium carbonate, and gamma-butyrolactone (Roy et al. 1985). Because of the simultaneous ingestion of other chemicals, the role of 4,4′-methylenedianiline, if any, in causing this respiratory effect cannot be ascertained.

No gross or histopathogical alterations were observed in the lungs, trachea, bronchi, or nasal cavity of rats administered up to 141 mg 4,4′-methylenedianiline/kg/day in drinking water for 13 weeks or up to 19 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). Similar findings were reported in mice administered up to 116 mg 4,4′-methylenedianiline/kg/day in the drinking water for 13 weeks or up to 57 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). An earlier study reported lung congestion and hyperemia, pneumonia, and pulmonary edema in female beagle dogs treated with doses of approximately 2.7 mg 4,4′-methylenedianiline/kg/day for 54-84 months (Deichmann et al. 1978). The test material was dissolved in corn oil and administered in a gelatin capsule 3 times per week. Because this study used only a total of 9 animals and no concurrent controls were-used, the validity of the findings is unclear.

Table 2-2. Levels of Significant Exposure to 4,4'-Methylenedianiline - Oral

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Death						
1	Rat (Wistar)	once (G)				830 (LD_{50})	Pludro et al. 1969
2	Rat (Wistar)	once (GO)				335 M (LD_{50})	Schmidt et al. 1980
3	Mouse (B6C3F1)	14 d (ad libitum) (W)				207 M (2/5 died) 220 F (1/5 died)	NTP 1983
4	Gn Pig (NS)	once (GO)				260 M (LD_{50})	Schmidt et al. 1974
5	Rabbit (NS)	once (GO)				620 M (LD_{50})	Schmidt et al. 1974
	Systemic						
6	Rat (Sprague-Dawley)	once (GO)	Hepatic		25[b] M (increased serum alanine aminotransferase and relative liver weight)	100 M (hepatocellular and bile duct necrosis and hemorrhage)	Bailie et al. 1993
7	Rat (Sprague-Dawley)	once (GO)	Hepatic			50 M (cholestasis, biliary epithelial injury, hepatic parenchymal damage)	Bailie et al. 1994
8	Rat (Sprague-Dawley)	once (G)	Gastro	250M			Kanz et al. 1992
			Hepatic			250 M (necrosis of bile duct epithelial cells, focal periportal hepatocellular necrosis)	

Table 2-2. Levels of Significant Exposure to 4,4'-Methylenedianiline - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
9	Rat (Fischer- 344)	14 d (ad libitum) (W)	Gastro	130 F 235 M		261 F 469 M (ulceration and hemorrhage in cardiac portion of stomach)	NTP 1983
			Bd Wt	57 M 65 F	117 M 130 F (10% lower final body weight)	235 M 261 F (31% lower final body weight)	
10	Rat (Wistar)	once (GO)	Hepatic			50 M (bile duct necrosis; increased serum AP, ALT, glutamate dehydrogenase, and leucine aminopeptidase)	Schmidt et al. 1980
11	Rat (Sprague-Dawley)	5-14 d 1x/d (GW)	Endocr		110 F (hypertrophy and lipid accumulation in adrenal cortex; increased thyroid weight and loss of colloid in thyroid follicles)		Tullner 1960
			Bd Wt		110 F (17% reduction in final body weight)		
12	Mouse (B6C3F1)	14 d (ad libitum) (W)	Bd Wt	207 M 220 F	415 M 441 F (15% reduction in final body weight)		NTP 1983
	Immunological/Lymphoreticular						
13	Rat (Sprague-Dawley)	once (G)			250M (disintegration of cortical thymocytes; cytolysis in cortical lymphocytes)		Kanz et al. 1992
	Reproductive						
14	Rat (Sprague-Dawley)	5-14 d 1x/d (GW)			110 F (increased weight of the uterus; atypical folliculoid endometrial response)		Tullner 1960

Table 2-2. Levels of Significant Exposure to 4,4'-Methylenedianiline - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	INTERMEDIATE EXPOSURE						
	Death						
15	Rat (Sprague-Dawley)	30 d 1 x/3d (GO)				36 F (16% decreased survival)	Griswold et al. 1968
	Systemic						
16	Rat (Wistar)	8-40 wk (F)	Hepatic		92M (hyperplasia of bile ducts, increased relative liver weight)		Fukushima et al. 1979
			Renal	92M			
			Bd Wt			92 M (44% reduction in weight gain after 40 weeks of treatment)	
17	Rat (Fischer-344)	34 wk (ad lib) (W)	Hepatic		88M (bile duct proliferation)		Fukushima et al. 1981
			Renal	88M			
			Endocr		88M (hyperplastic goiter)		
			Bd Wt			88 M (40% reduction in body weight gain)	
18	Rat (Fischer-344)	32 wk (ad lib) (F)	Hepatic		100M (bile duct proliferation)		Fukushima et al. 1981
19	Rat (Fischer-344)	8 wk (F)	Hepatic		100M (bile duct proliferation; fatty changes)		Hagiwara et al. 1993
			Endocr		100M (hyperplastic goiter of the thyroid)		

Table 2-2. Levels of Significant Exposure to 4,4'-Methylenedianiline - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
20	Rat (Inbred W)	19 wk (F)	Endocr		84 M (thyroid hyperplasia)		Hiasa et al. 1984
			Bd Wt			79 M (27% reduction in body weight gain)	
21	Rat (Wistar)	12 wk (F)	Hepatic			84 M (bile duct proliferation; fatty infiltration; fibrosis; increased SGOT, SGPT, AP, and BSP retention)	Miyamoto et al. 1977
			Bd Wt			84 M (58% reduction in body weight gain)	
22	Rat (Fischer- 344)	13 wk (ad libitum) (W)	Resp	141 F			NTP 1983
			Cardio	141 F			
			Gastro	141 F			
			Musc/skel	141 F			
			Hepatic	33 M 35 F	67 M (bile duct hyperplasia in 70 F 4/10 males and 3/10 females)		
			Renal	141 F			
			Endocr	33 M 35 F	67 M (hyperplastic goiter in 70 F 3/10 males and 1/10 females)		
			Dermal	141 F			
			Bd Wt	67 M 70 F		134 M (21% reduced final body 141 F weight in males, 26% in females)	

Table 2-2. Levels of Significant Exposure to 4,4'-Methylenedianiline - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
23	Rat (Wistar)	12 wk 1 x/d (G)	Gastro	8.3		83 (intestinal occlusion)	Pludro et al. 1969
			Hemato	83			
			Hepatic	8.3[c]	83 (atrophy of hepatocytes; increased relative liver weight)		
			Renal	83			
			Bd Wt	83			
24	Rat (Fischer- 344)	24 wk (F)	Endocr		97M (hyperplastic goiter)		Tsuda et al. 1987
			Bd Wt			97 M (70% reduction in body weight gain)	
25	Mouse (B6C3F1)	13 wk (ad libitum) (W)	Resp	116 F			NTP 1983
			Cardio	116 F			
			Gastro	116 F			
			Musc/skel	116 F			
			Hepatic	58 F	108M (bile duct hyperplasia in 5/10)		
			Renal	116 F			
			Endocr	116 F			
			Dermal	116 F			
			Bd Wt	54 M 116 F	108M (13% reduction in final body weight)		
	Immunological/Lymphoreticular						
26	Rat (Fischer- 344)	13 wk (ad libitum) (W)		141 F			NTP 1983

Table 2-2. Levels of Significant Exposure to 4,4'-Methylenedianiline - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
27	Mouse (B6C3F1)	13 wk (ad libitum) (W)		116 F			NTP 1983
	Neurological						
28	Rat (Fischer- 344)	13 wk (ad libitum) (W)		141 F			NTP 1983
29	Mouse (B6C3F1)	13 wk (ad libitum) (W)		116 F			NTP 1983
	Reproductive						
30	Rat (Fischer- 344)	13 wk (ad libitum) (W)		141 F			NTP 1983
31	Mouse (B6C3F1)	13 wk (ad libitum) (W)		116 F			NTP 1983

Table 2-2. Levels of Significant Exposure to 4,4'-Methylenedianiline - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	CHRONIC EXPOSURE						
	Death						
32	Mouse (B6C3F1)	103 wk (ad libitum) (W)				57 M (20% reduced survival rate)	Lamb et al. 1986; NTP 1983
	Systemic						
33	Rat (Fischer- 344)	103 wk (ad libitum) (W)	Resp	19 F			Lamb et al. 1986; NTP 1983
			Cardio	19 F			
			Gastro	19 F			
			Musc/skel	19 F			
			Hepatic		9M (fatty metamorphosis, focal cellular change)		
			Renal	9 M 19 F	16M (mineralization of the kidney)		
			Endocr	10 F	19 F (follicular cysts in thyroid; follicular cell hyperplasia)		
			Dermal	19 F			
			Bd Wt	19 F			

Table 2-2. Levels of Significant Exposure to 4,4'-Methylenedianiline - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
34	Mouse (B6C3F1)	103 wk (ad libitum) (W)	Resp	57 M			Lamb et al. 1986; NTP 1983
			Cardio	57 M			
			Gastro	57 M			
			Musc/skel	57 M			
			Hepatic		25 M (liver degeneration)		
			Renal			25 M (nephropathy) 19 F	
			Endocr	25 M	57 M (thyroid cell hyperplasia) 43 F		
			Dermal	57 M			
			Bd Wt	25 M	57 M (13-16% reduction in 43 F final body weight)		
	Immunological/Lymphoreticular						
35	Rat (Fischer- 344)	103 wk (ad libitum) (W)		19 F			NTP 1983; Lamb et al. 1986
36	Mouse (B6C3F1)	103 wk (ad libitum) (W)		57 M			NTP 1983; Lamb et al. 1986
	Neurological						
37	Rat (Fischer- 344)	103 wk (ad libitum) (W)		19 F			NTP 1983; Lamb et al. 1986
38	Mouse (B6C3F1)	103 wk (ad libitum) (W)		57 M			NTP 1983; Lamb et al. 1986

Table 2-2. Levels of Significant Exposure to 4,4'-Methylenedianiline - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less serious (mg/kg/day)		Serious (mg/kg/day)	
	Reproductive							
39	Rat (Fischer- 344)	103 wk (ad libitum) (W)		19 F				Lamb et al. 1986; NTP 1983
40	Mouse (B6C3F1)	103 wk (ad libitum) (W)		57 M				NTP 1983; Lamb et al. 1986
	Cancer							
41	Rat (Fischer- 344)	103 wk (ad libitum) (W)					9 M, 10 F (CEL: increased incidence of neoplastic nodules in liver)	Lamb et al. 1986; NTP 1983
42	Mouse (B6C3F1)	103 wk (ad libitum) (W)					19 F (CEL: malignant lymphoma and adenoma/carcinoma of the liver)	Lamb et al. 1986; NTP 1983

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

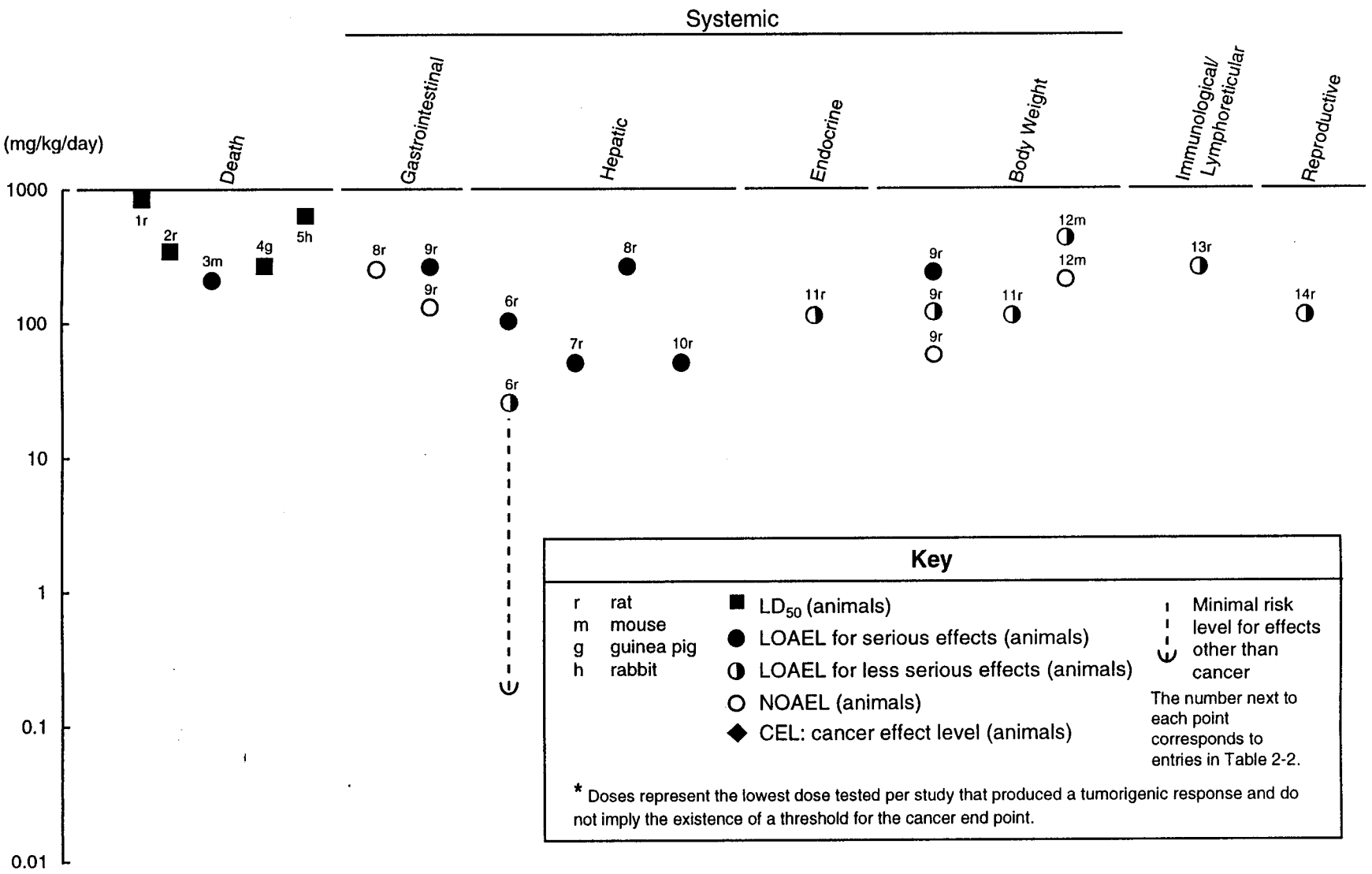
[b]Used to derive an acute oral Minimal Risk Level (MRL) of 0.2 mg/kg/day; unadjusted dose divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL,10 for extrapolation from rats to humans, and 10 for human variability). A modifying factor of 0.5 was used to account for facilitated absorption by the corn oil vehicle.

[c]Used to derive an intermediate-duration oral MRL of 0.08 mg/kg/day; unadjusted dose divided by an uncertainty factor of 100 (10 for extrapolation from rats to humans and 10 for human variability)

ALT = alanine amino transferase; AP = alkaline phosphatase; Bd Wt = body weight; BSP = bromosulphalein; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Derm = dermal; Endocr = endocrine; F = female; (F) = feed; (G) = gavage; Gastro = gastrointestinal; (GO) = gavage (oil); (GW) = gavage (water); Hemato = hematological; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; (W) = water; wk = week(s); x = time(s)

Figure 2-2. Levels of Significant Exposure to 4,4-Methylenedianiline - Oral

Acute (≤14 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 end point.

Figure 2-2. Levels of Significant Exposure to 4,4-Methylenedianiline - Oral (cont.)

Intermediate (15-364 days)

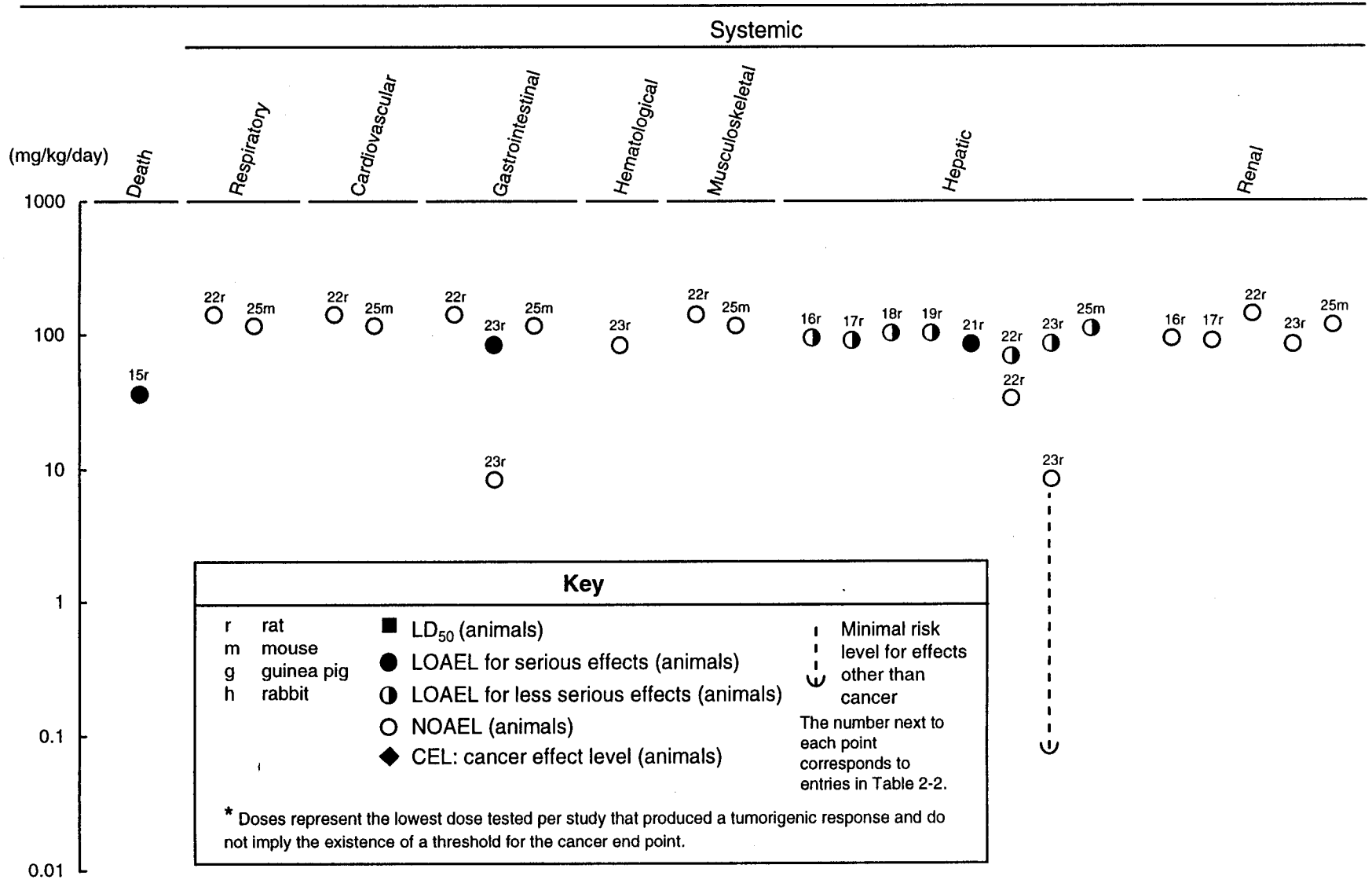


Figure 2-2. Levels of Significant Exposure to 4,4-Methylenedianiline - Oral (cont.)

Intermediate (15-364 days)

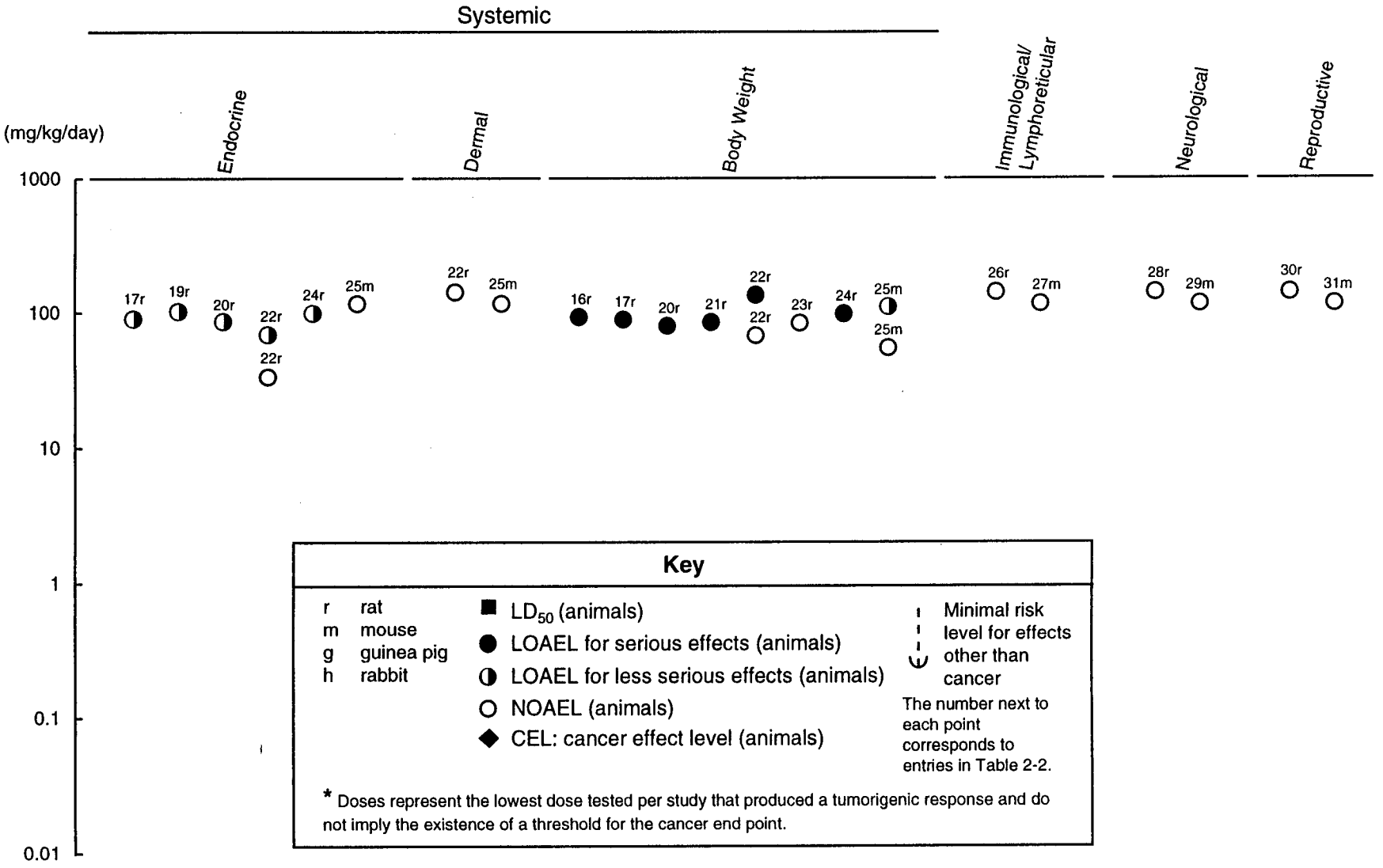
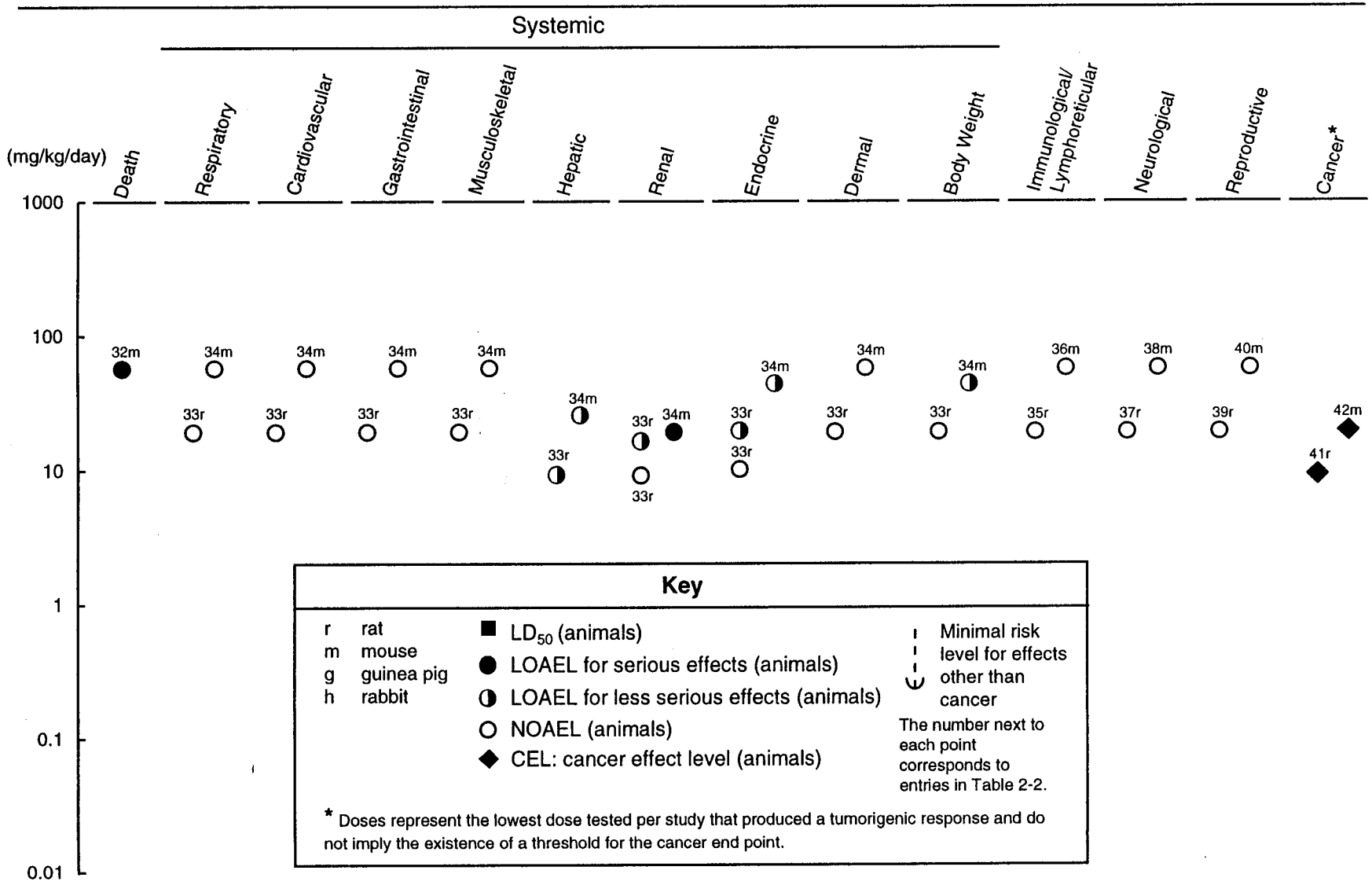


Figure 2-2. Levels of Significant Exposure to 4,4-Methylenedianiline - Oral (cont.)

Chronic (≥365 days)



Cardiovascular Effects. Very limited and inconclusive information exists regarding cardiovascular effects in humans after ingestion of 4,4′-methylenedianiline. Bradycardia, hypotension, and abnormal electrocardiogram were reported in a male subject who accidentally drank an undetermined amount of a solution containing 4,4′-methylenedianiline, potassium carbonate, and gamma-butyrolactone (Roy et al. 1985). These signs were observed on arrival to the hospital shortly after the accident occurred. Because of the simultaneous ingestion of other chemicals, the role of 4,4′-methylenedianiline, if any, cannot be determined.

Information on effects in animals is also limited. No gross or histopathogical alterations were observed in the heart of rats administered up to 141 mg 4,4′-methylenedianiline/kg/day in drinking water for 13 weeks or up to 19 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). Similar findings were reported in mice administered up to 116 mg 4,4′-methylenedianiline/kg/day in the drinking water for 13 weeks or up to 57 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983).

Gastrointestinal Effects. Nausea, abdominal pain, and vomiting were reported in one female and five males (ages 17-25) who drank an alcoholic beverage spiked with 4,4′-methylenedianiline (Tillmann et al. 1997); the amount of 4,4′-methylenedianiline ingested was not known. No further information was located regarding gastrointestinal effects in humans following oral exposure to 4,4′-methylenedianiline.

Doses of ≥261 mg 4,4′-methylenedianiline/kg/day administered in the drinking water for 14 days induced crater-like foci in the cardiac portion of the stomach in female rats (NTP 1983). The NOAEL for this effect in females was 130 mg/kg/day. No such lesions were seen in males treated with up to 235 mg/kg/day for the same period of time (NTP 1983), but lesions were evident at 469 mg/kg/day. Longer-duration studies reported no gross or histopathogical alterations in the salivary glands, esophagus, stomach, pancreas, duodenum, jejunum, ileum, and colon from rats administered up to 141 mg 4,4′-methylenedianiline/kg/day in drinking water for 13 weeks or up to 19 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). Similar findings were reported in mice administered up to 116 mg 4,4′-methylenedianiline/kg/day in the drinking water for 13 weeks or up to 57 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). Intestinal occlusion was reported in an earlier study in rats treated by gavage with 83 mg 4,4′-methylenedianiline/kg/day for 12 weeks; the NOAEL was 8.3 mg/kg/day (Pludro et al. 1969). No further information was provided in this study.

Hematological Effects. Limited information was located regarding hematological effects in humans after oral exposure to 4,4′-methylenedianiline. A male subject developed eosinophilia with left shift in neutrophils 7-35 days after accidentally ingesting a solution containing a 4,4′-methylenedianiline, potassium carbonate, and gamma-butyrolactone (Roy et al. 1985). This is consistent with the appearance of erythema multiform which is characterized by eosinophilia. A recent study reports that an 18-year-old male had mild leucocyte elevation 1 day after drinking an alcoholic beverage spiked with 4,4′-methylenedianiline (Tillmann et al. 1997); his blood cell count and thrombocyte rate were normal. The amount of 4,4′-methylenedianiline ingested was not known.

The data in animals are limited to a study that reported no alterations in hemoglobin levels or erythrocyte counts in rats treated daily by gavage for 12 weeks with doses of 83 mg 4,4′-methylenedianiline/kg/day (Pludro et al. 1969).

Musculoskeletal Effects. One female and five males (ages 17-25) complained of muscle and joint pain after drinking an alcoholic beverage spiked with 4,4′-methylenedianiline (Tillmann et al. 1997). No further information was located.

Very limited information was found regarding musculoskeletal effects of 4,4′-methylenedianiline in animals after oral exposure. No gross or histopathogical alterations were observed in thigh muscle and costochondral junction (rib) of rats administered up to 141 mg 4,4′-methylenedianiline/kg/day in drinking water for 13 weeks or up to 19 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). Similar findings were reported in mice administered up to 116 mg 4,4′-methylenedianiline/kg/day in the drinking water for 13 weeks or up to 57 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983).

Hepatic Effects. A local outbreak of jaundice, which was later traced to ingestion of 4,4′-methylenedianiline, occurred in the Epping district of Essex, England, in 1965 (Kopelman et al. 1966). Eighty-four subjects became ill shortly after eating bread prepared with flour that had been contaminated with 4,4′-methylenedianiline. Three general types of clinical presentations were observed. In a majority of the patients, the illness had an acute onset with severe intermittent pain in the upper abdomen and lower chest for 24-36 hours. Over the next few days, the patients in this group improved, but then developed a flu-like condition with fever and increasing jaundice. The liver was enlarged and tender. After a few days, the liver became smaller, but the jaundice persisted for weeks. These patients did not feel completely recovered for a considerable period of time. A second

group of patients only showed mild symptoms of upper abdominal discomfort. A week later, however, they too developed fever and increasing jaundice, which persisted longer than in the first group. A third group, with the least common symptoms, had severe jaundice when first seen, but had minimal preceding symptoms. The liver in these patients was often enlarged, but rarely tender. Clinical chemistry tests showed increases in serum bilirubin, alkaline phosphatase activity, and glutamic oxaloacetic transaminase. Needle biopsy performed in 4 cases within 2-3 weeks of the onset of symptoms showed cellular infiltration and cholestasis, and there was evidence of damage to the liver parenchyma and biliary tree. This, according to the investigators (Kopelman et al. 1966), is the first documented case of human poisoning with 4,4′-methylenedianiline. Forty-three of these subjects were evaluated 2 years later (Kopelman 1968). Aside from slight abnormalities in single liver tests and complaints of subjective nature, there was no evidence of progressive hepatic disease.

Liver toxicity was also observed in the case of a male subject who accidentally drank an unspecified amount of a solution containing 4,4′-methylenedianiline, potassium carbonate, and gamma-butyrolactone (Roy et al. 1985). Clinical tests conducted 2 days after admission to the hospital showed elevated transaminases and hyperbilirubinemia. Slight hepatomegaly developed 6 weeks after admission. One year after the accident, serum transaminases still had not returned to normal levels. Although other chemicals were involved in this case, the signs and symptoms are consistent with those reported by Kopelman et al. (1966) and were most likely caused by 4,4′-methylenedianiline. A case of a young man who drank an alcoholic beverage spiked with 4,4′-methylenedianiline and developed liver toxicity was recently described in the literature (Tillmann et al. 1997). The subject thought that the substance was methylenedioxyamphetamine, a psychoactive drug. Upon admission to the hospital, 24 hours after drinking the beverage, his bilirubin and liver enzyme activities were elevated and increased steadily over 7 days; he also developed jaundice. By the time he was discharged on day 15, the jaundice had vanished and the bilirubin was close to normal. Tillmann et al. (1997) indicates that five other subjects, who also drank the spiked beverage, developed a similar picture of liver toxicity.

Numerous studies of various durations have demonstrated that the liver is a target for 4,4′ methylene-dianiline toxicity in animals, particularly in rats. A single gavage dose of 25 mg/kg (range tested 25-225 mg/kg) increased serum alanine aminotransferase activity and relative liver weight in rats (Bailie et al. 1993). Higher single doses (50-250 mg/kg) induced cholestasis, biliary epithelial injury, bile duct necrosis, and periportal hepatocellular necrosis (Bailie et al. 1993, 1994; Kanz et al. 1992; Schmidt et al. 1980). The earliest change identified was bile ductular necrosis 4 hours after dosing (Bailie et al. 1993). These single-

dose studies demonstrated that 4,4′-methylenedianiline is selectively toxic to bile duct in rats and that hepatic lesions appear after the lesions to the bile ducts (Kanz et al. 1992). The minimal effective dose of 25 mg/kg from the Bailie et al. (1993) study is considered a minimal LOAEL and is the basis for derivation of an acute oral MRL of 0.2 mg/kg/day.

Results from intermediate-duration studies support those from single-dose studies. LOAEL values in the range of 67-100 mg 4,4′-methylenedianiline/kg/day have been identified in rats (Fukushima et al. 1979, 1981; Hagiwara et al. 1993; Miyamoto et al. 1977; NTP 1983; Pludro et al. 1969). However, in many of these studies only one dose level was used. Exceptions are the NTP (1983) study in which NOAELs of 35 mg/kg/day and 58 mg/kg/day were identified in rats and mice, respectively, and the earlier report by Pludro et al. (1969) that established a NOAEL of 8.3 mg/kg/day in rats. This NOAEL, 8.3 mg/kg/day, served as the basis for derivation of an intermediate oral MRL of 0.08 mg/kg/day. Gavage, drinking water, or diet were used as vehicles in the studies mentioned above, which suggests that the method of administration of 4,4′-methylenedianiline is not a determining factor in liver toxicity. In addition to elevated serum transaminases, the most commonly seen liver alterations were hyperplasia of the bile ducts, fatty infiltration, fibrosis, and atrophy of the liver parenchyma. In general, it appeared that most of the hepatic lesions were at least partially reversible following cessation of treatment.

Liver dilation, fatty metamorphosis, and focal cellular change were described in rats treated with 9 mg 4,4′-methylenedianiline/kg/day in the drinking water for 103 weeks (Lamb et al. 1986; NTP 1983). In the same study, liver degeneration was seen in mice receiving 25 mg/kg/day. Both dose levels represent the lowest levels tested. In a study in dogs, all 9 of the treated animals had liver lesions that included hepatic cell necrosis, fatty infiltration, and portal fibrosis after treatment with approximately 2.7 mg 4,4′-methylenedianiline/kg/day, 3 days per week for 54-84 months (Deichmann et al. 1978). Because of study limitations, such as a small number of animals used and lack of concurrent controls, it is not possible to conclusively determine whether dogs are more sensitive than rodents.

Renal Effects. Only one report was located that described adverse renal effects in humans following oral exposure to 4,4′-methylenedianiline. In this case report (Roy et al. 1985), a male subject accidentally drank an unspecified amount of a liquid containing 4,4′-methylenedianiline, potassium carbonate, and gamma-butyrolactone. Tests conducted two days after the accident showed hematuria and glycosuria. In the presence of normoglycemia, glycosuria indicated renal tubular

dysfunction. Because there was simultaneous ingestion of other chemicals, the role of 4,4′-methylenedianiline, if any, cannot be conclusively determined. Proteinuria and erythrocyturia were described in a young man who drank an alcoholic beverage spiked with 4,4′-methylenedianiline (Tillmann et al. 1997). No further information was located.

Several reports have investigated renal effects of 4,4′-methylenedianiline in animals after intermediateand chronic-duration exposure. No gross or histopathological alterations have been reported in the kidneys or urinary bladder of rats treated with 4,4′-methylenedianiline in the range of 83-141 mg/kg/day for periods ranging between 8-40 weeks (Pukushima et al. 1979, 1981; NTP 1983; Pludro et al. 1969). Administration vehicles included gavage, drinking water, and diet. Similar lack of effects were reported in mice treated with up to 116 mg 4,4′-methylenedianiline/kg/day in the drinking water for 13 weeks (NTP 1983). In contrast, high incidence of kidney mineralization was observed in male rats treated for 103 weeks with dose of 16 mg 4,4′-methylenedianiline/kg/day in the drinking water (Lamb et al. 1986; NTP 1983). No such effect was observed in females treated with a similar dose (Lamb et al. 1986; NTP 1983). A high incidence of nephropathy was reported in male and female mice treated with 19-25 mg 4,4′methylenedianiline/kg/day in the drinking water for 103 weeks (Lamb et al. 1986; NTP 1983). Male mice also exhibited renal papilla mineralization at a dose level of 57 mg/kg/day (Lamb et al. 1986; NTP 1983). Various lesions to the kidney and urinary bladder were observed in 9 dogs treated with approximately 2.7 mg 4,4′-methylenedianiline/kg/day, 3 days per week for 54-84 months (Deichmann et al. 1978). Kidney abnormalities included rough surface, congestion, glomerulonephritis, cloudy swellin,, and thickening of the basement membrane. Hyperemia in the urinary bladder was noticed in two dogs, whereas mucosal hyperplasia, edema, lymphocytic infiltration, and marked congestion of the urinary bladder were observed in another dog. This study has severe limitations, such as a very small number of animals (9 female beagle dogs), no concurrent controls, and only one dose level was tested; therefore, the results must be interpreted with caution.

Endocrine Effects. No studies were located regarding endocrine effects in humans after oral exposure to 4,4′-methylenedianiline.

Numerous studies in rats have identified the thyroid as a sensitive organ for 4,4′-methylenedianiline toxicity. Hypertrophy and histopathological alterations of the adrenals and thyroid were reported in rats administered doses of 1l0-146 mg 4,4′-methylenedianiline/kg/day by gavage for 5- 14 days

(Tullner 1960). Longer duration studies (8-32 weeks) in rats have established LOAELs for thyroid effects in the range of 67-100 mg 4,4′-methylenedianiline/kg/day (Fukushima et al. 1981; Hagiwara et al. 1993; Hiasa et al. 1984; NTP 1983; Tsuda et al. 1987). The administration vehicle was drinking water or food. With the exception of the NTP (1983) study, only one dose level was tested in these studies. In the NTP (1983) report, a NOAEL of 35 mg/kg/day was identified for rats and 116 mg/kg/day for mice; the latter was the highest dose level tested in mice. The thyroid alterations observed consisted of hyperplasia, decrease of colloid in the follicles, reduced follicle size, and slight reduction in serum T_3 and T_2. In addition to thyroid effects, pituitary basophile hypertrophy was noticed in male and female rats that received approximately 140 mg 4,4′-methylenedianiline/kg/day in the drinking water for 13 weeks (NTP 1983). Results from chronic-duration studies revealed follicular cysts and follicular cell hyperplasia in the thyroid of female rats treated with 19 mg 4,4′-methylenedianiline/kg/day in the drinking water for 103 weeks and thyroid cell hyperplasia in male and female mice treated in the same manner with 57 mg/kg/day and 43 mg/kg/day, respectively (Lamb et al.1986; NTP 1983).

The overall evidence suggests that thyroid, particularly in rats, may be a sensitive organ for 4,4′-methylenedianiline toxicity. However, until other animal species are tested, it is uncertain whether rats are the most sensitive species.

Dermal Effects. Limited relevant dam in humans were located. Roy et al. (1985) described the case of a man who developed erythema multiform after accidentally ingesting a solution containing 4,4′-methylenedianiline, potassium carbonate, and gamma-butyrolactone (Roy et al. 1985). This finding is consistent with an allergic reaction to 4,4′-methylenedianiline (see Section 2.2.3). In a recent case report, an 18-year-old male developed a skin rash 7 days after drinking an alcoholic beverage spiked with 4,4′-methylenedianiline (Tillmann et al. 1997). The rash had cleared by the time he was discharged from the hospital on day 15. No further information was located.

Very limited information was found regarding dermal effects of 4,4′-methylenedianiline in animals after oral exposure. No gross or histopathogical alterations were observed in the skin of rats administered up to 141 mg 4,4′-methylenedianiline/kg/day in drinking water for 13 weeks or up to 19 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). Similar findings were reported in mice administered up to 116 mg 4,4′-methylenedianiline/kg/day in the drinking water for 13 weeks or up to 57 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983).

Ocular Effects. Ocular effects were described in the case of a male subject who accidentally ingested an unspecified amount of a solution containing 4,4′-methylenedianiline, potassium carbonate, and gamma-butyrolactone (Roy et al. 1985). Four days after admission to the hospital, his vision became blurred and visual acuity was reduced considerably. This condition worsened on subsequent weeks and he developed a coarse pigmentary retinopathy similar to that of retinitis pigmentosa. Tests conducted later revealed gross malfunction of the retinal pigment epithelium, a condition which improved little over the next 18 months. Although the subject ingested a mixture of three components, the retina is not known to be a target for gamma-butyrolactone or potassium carbonate toxicity. Nevertheless, there is no conclusive evidence that the effects observed were caused by 4,4′-methylenedianiline.

No studies were located regarding ocular effects in animals after oral exposure to 4,4′-methylenedianiline.

Body Weight Effects. No studies were located regarding body weight effects in humans following oral exposure to 4,4′-methylenedianiline.

Oral exposure to 4,4′-methylenedianiline in animals usually resulted in dose-related reduction in body weight gain, and occasionally, weight loss. Male rats exposed for 14 days in the drinking water to 235 mg 4,4′-methylenedianiline/kg/day had a 31% reduction in final body weight relative to untreated controls (NTP 1983). At the lowest dose level tested, 117 mg/kg/day in males and 130 mg/kg/day in females, final body weights were reduced by about 11%. In mice treated in the same manner, the NOAEL and LOAEL for body weight effects was 220 mg/kg/day and 415 mg/g/day, respectively (NTP 1983). Numerous intermediate-duration studies (8-40 weeks) have reported decreased body weight gain in rats treated with 4,4′-methylenedianiline doses in the range of 84 mg/kg/day to 141 mg/kg/day (Fukushima et al. 1979, 1981; Hagiwara et al. 1993; Hiasa et al. 1984; Miyamoto et al. 1977; NTP 1983; Tsuda et al. 1987). The administration vehicle varied between food and drinking water. In these studies, final body weights were reduced 27-70% relative to untreated controls. The sole exception is a report by Pludro et al. (1969) which identified a NOAEL of 83 mg/kg/day in rats treated by gavage for 12 weeks and no explanation is apparent for this discrepancy. In the studies mentioned above, except for the NTP (1983) study, only one dose level of 4,4′-methylenedianiline was tested. The NOAEL for rats in the NTP (1983) report was 70 mg/kg/day. In mice, the NOAEL and

LOAEL were 54 mg/kg/day and 108 mg/kg/day, respectively (NTP 1983). None of the studies mentioned above provided data regarding food consumption.

Body weights from rats treated for 103 weeks with up to 19 mg 4,4′-methylenedianiline/kg/day in the drinking water were not significantly different than untreated controls (Lamb et al. 1986; NTP 1983). However, in the same study (Lamb et al. 1986; NTP 1983), final body weight was reduced by 13% in male mice treated with 57 mg 4,4′-methylenedianiline/kg/day and by 16% in females treated with 43 mg 4,4′-methylenedianiline/kg/day. The NOAEL was about 20 mg/kg/day. Again, food consumption data were not provided.

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after oral exposure to 4,4′-methylenedianiline.

Data in animals are restricted to histopathological examinations of organs of the lymphoreticular system, but no information is available regarding possible effects on immunocompetence. Focal disintegration of cortical thymocytes was observed in rats 8 hours after a single gavage dose of 250 mg 4,4′-methylenedianiline/kg (Kanz et al. 1992). In some rats, approximately 50% of the thymus cortex was necrotic 24 hours after dosing. However, no histopathological alterations were observed in the spleen (Kanz et al. 1992). A 13-week drinking water study reported no histopathological alterations in the spleen, thymus, and lymph nodes of rats treated with up to 141 mg 4,4′-methylenedianiline/kg/day (NTP 1983). The same findings were reported in mice treated in the same manner with up to 116 mg 4,4′-methylenedianilinelkglday (NTP 1983). The results from the NTP (1983) study are in conflict with those of Pludro et al. (1969) who reported unspecified lesions in the spleen in rats treated with daily gavage doses of 8.3 mg 4,4′-methylenedianiline/kg. Doses of 83 mg/kg/day induced hyperplasia in lymphatic nodes. A possible explanation for the discrepancy between the results from these two studies is the use of different administration vehicles, drinking water in the NTP (1983) and gavage in propylene glycol in the Pludro et al. (1969) study.

There were no histopathological changes in the spleen, thymus, and lymph nodes from rats treated for 103 weeks with up to 19 mg 4,4′-methylenedianiline/kg/day in the drinking water (Lamb et al. 1986; NTP 1983). Similar findings were reported in mice treated with up to 57 mg 4,4′-methylene-

dianiline/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). In dogs treated for 54-84 months wit1 approximately 2.7 mg 4,4´-methylenedianiline/kg/day (dose given 3 times/week by gavage in a capsule) the spleens appeared shrunken and the surface had a granular appearance (Deichmann et al. 1978). These latter investigators also reported splenitis with thickening and hyalinization of the capsule, trabeculae and lymphoid corpuscles. Hemosiderosis and spleen congestion, which were also noticed, may have been secondary to hematological effects (not reported) such as hemolytic anemia and methemoglobinemia. This study in dogs was poorly conducted and poorly reported; thus, it is unclear whether dogs are a particularly sensitive species.

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

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to 4,4´-methylenedianiline.

Information on neurological effects in animals is very limited. No gross or histopathogical alterations were observed in the sciatic nerve, brain, and spinal cord of rats administered up to 141 mg 4,4´-methylenedianiline/kg/day in drinking water for 13 weeks or up to 19 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). Similar findings were reported in mice administered up to 116 mg 4,4´-methylenedianiline/kg/day in the drinking water for 13 weeks or up to 57 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). No further neurological parameters were evaluated. The limited information available suggests that 4,4´-methylenedianiline is not a neurotoxicant. The highest NOAEL values and all reliable LOAEL values for neurological effects for each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to 4,4´-methylenedianiline.

Very little information exists regarding reproductive effects of 4,4′-methylenedianiline in animals. An acute-duration study reported a 71% increase in absolute weight of the uterus in ovariectomized rats administered 1l0-146 mg 4,4′-methylenedianiline by gavage for 5-14 days (Tullner 1960). Histopathological examination of the uterus revealed an atypical folliculoid response in the endometrium. No further information was provided in this study. No gross or histopathogical alterations were observed in the ovaries, uterus, mammary glands, seminal vesicles, prostate, or testes of rats administered up to 141 mg 4,4′-methylenedianiline/kg/day in drinking water for 13 weeks or up to 19 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). Similar findings were reported in mice administered up to 116 mg 4,4′-methylenedianiline/kg/day in the drinking water for 13 weeks or up to 57 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). No further reproductive parameters were evaluated. The limited information available is insufficient to determine whether exposure to 4,4′-methylenedianiline may alter reproductive function.

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

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to 4,4′-methylenedianiline. Only one report was located that provided information on effects in animals. In that study (Bourdelat et al. 1983), fetuses from pregnant rats treated by gavage with 37 mg 4,4′-methylenedianiline/kg/day (as the chlorohydrate) on gestation days 14-20 had liver alterations in the form of fatty infiltration of the parenchyma. This dose level also caused histological alterations in the livers from the dams. Fetuses from 1 of 5 dams administered 219 mg 4,4′-methylenedianiline/ kgjday on gestation days 7-20 showed delayed closing of the calvaria, enlarged tongue, and an abnormally large snout (Bourdelat et al. 1983). The 219 mg/kg/day dose level was lethal to 1 of 5 pregnant rats. Because of study limitations such as the use of only one female rat as control and lack of detailed reporting of the results, this study is not presented in Table 2-2. -

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after oral exposure to 4,4′-methylenedianiline.

Administration of 4, 20, or 50 mg 4,4′-methylenedianiline/kg/day by gavage for 3 days to male Fischer 344 rats resulted in the dose-related formation of adducts with liver DNA, as detected by 32Ppostlabeling analysis (Vock et al. 1996). However, DNA isolated from the bladder and from lymphocytes from these animals did not show any treatment-related DNA-adducts.

Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

A recent study examined the causes of death (neoplastic and non-neoplastic diseases) in a population that had consumed bread contaminated with 4,4′-methylenedianiline in 1965 in the Epping district of Essex, England (Hall et al. 1992). Liver toxicity was the main adverse effect reported at the time of the accident (Kopelman et al. 1966). Of the original 84 cases, 55 people were alive, 18 had died, and 16 could not be traced. Of those alive, 58% completed a health questionnaire. The causes of death were, by and large, unremarkable, with the possible exception of one case of biliary duct carcinoma. The observed/expected ratios for cancer and non-neoplastic diseases were well below 1.0. The results suggested that there was no obvious link between current health status and the poisoning episode. The one case of biliary duct carcinoma was of interest because this tumor, according to the investigators (Hall et al. 1992), is very rare in humans.

The carcinogenic potential of oral administration of 4,4′-methylenedianiline has been examined in dogs (Deichmann et al. 1978), rats, and mice (Griswold et al. 1968; Lamb et al. 1986; NTP 1983). No significant carcinogenic response was reported in rats treated for 30 days with 36 mg 4,4′-methylenedianiline/kg/day by gavage and observed for 9 months (Griswold et al. 1968); however, the observation period may have been too short. Dogs that received 5-6.26 grams 4,4′-methylenedianilinelkg for a period of 54-84 months (the time-weighted dose can be estimated at about 2.7 mg/kg/day) did not show a significant increase in bladder or liver tumors (Deichmamr et al. 1978).
This study has severe limitations such as a very small number of animals (9 female beagle dogs), no concurrent controls, and practically only one dose level was tested; therefore, the results must be interpreted with caution. In a 2-year bioassay (Lamb et al. 1986; NTP 1983), Fischer 344 rats were treated with 4,4′-methylenedianiline (as the dihydrochloride) in the drinking water. Doses were 9 and 16 mg/kg/day in males and 10 and 19 mg/kg/day in females. Clear evidence of carcinogenicity was found. In male rats, the incidence of follicular cell carcinomas of the thyroid gland was 0 of

49 (controls), 0 of 47 (low dose), and 7 of 48 (high dose); the incidence of neoplastic nodules of the liver was 1 of 50 (controls), 12 of 50 (low dose), and 25 of 50 (high dose). In females, the incidence of follicular cell adenomas was 0 of 47 (controls), 2 of 47 (low dose), and 17 of 48 (high dose); the incidence of C-cell adenomas was 0 of 47 (controls), 3 of 47 (low dose), and 6 of 48 (high dose). Tumors were also found in other tissues, but the increased incidence was not statistically significant.

4,4′-Methylenedianiline was also carcinogenic in B6C3F_1 mice when administered in the drinking water for 103 weeks (Lamb et al. 1986; NTP 1983). The doses were 25 and 57 mg/kg/day for males and 19 and 43 mg/kg/day for females. In males, the incidence of follicular adenomas of the thyroid gland was 0 of 47 (controls), 3 of 49 (low dose), and 16 of 49 (high dose); carcinomas of the liver occurred at an incidence of 10 of 49 (controls), 33 of 50 (low dose), and 29 of 50 (high dose). Also in males, the incidence of pheochromocytoma of the adrenal gland was 2 of 48 (controls), 12 of 49 (low dose), and 14 of 49 (high dose). In females, the following significant increased incidences were observed: follicular cell adenomas of the thyroid gland (0 of 50 controls, 1 of 47 low dose, 13 of 50 high dose), carcinomas/adenomas of the liver (4 of 50 controls, 15 of 50 low dose, 23 of 50 high dose), malignant lymphoma (13 of 50 controls, 28 of 50 low dose, 29 of 50 high dose), and alveolar/bronchiolar adenoma (1 of 50 controls, 2 of 50 low dose, 6 of 49 high dose).

Several studies have examined the effects of 4,4′-methylenedianiline on post-initiation stage carcinogenicity in various organs in rats. For example, administration of 100 mg 4,4′-methylenedianiline/kg/day in the diet for 32 weeks following initiation with N-ethyl-N-hydroxyethylnitrosamine reduced the incidence of hyperplastic nodules in the liver and neoplastic nodules in the kidney induced by the nitrosamine alone (Fukushima et al. 1981). Similar findings were reported regarding neoplastic responses in the urinary bladder when 4,4′-methylenedianiline (88 mg/kg/day for 34 weeks) followed initiation with N-butyl-N-(4-hydroxybutyl) nitrosamine (Fukushima et al. 1981), and regarding hepatocellular carcinomas when 4,4′-methylenedianiline (100 mg/kg/day for 26 weeks) followed initiation with diethylnitrosamine plus 2-acetylamino fluorene (Masui et al. 1986). In these studies, 4,4′-methylenedianiline alone was not carcinogenic. In contrast with the results summarized above, the incidence of thyroid tumors in rats initiated with N-bis(2-hydroxypropyl)nitrosamine and then treated with 4,4′-methylenedianiline (84 mg/kg/day for 19 weeks) was significantly higher (90%) than in rats treated only with the initiator (28%) (Hiasa et al. 1984); no tumors were seen in rats treated with 4,4′-methylenedianiline alone.

In a different type of experiment, administration of 100 mg 4,4′-methylenedianiline/kg/day for 8 weeks to rats followed by treatment with a combination of 3 carcinogens for 4 weeks resulted in a lower incidence of follicular cell hyperplasia and adenomas of the thyroid relative to rats treated with only the carcinogens (Hagiwara et al. 1993). The incidence of preneoplastic/neoplastic lesions observed in other tissues and organs was similar in the two groups.

The long-term bioassays conducted in rats and mice (Lamb et al. 1986; NTP 1983) provide clear evidence of 4,4′-methylenedianiline carcinogenicity in rodents. However, no evidence of carcinogenicity was found in intermediate-duration studies in the groups of rats that were treated with 4,4′-methylenedianiline alone (Fukushima et al. 1981; Hiasa et al. 1984; Masui et al. 1986). These are not necessarily inconsistent results, since exposure duration and observation periods may have been too short in the intermediate-duration studies.

The dose of levels of 9 mg 4,4′-methylenedianiline/kg/day for male rats and 19 mg/kg/day for female mice from the Lamb et al. (1986) and NTP (1983) studies are listed as Cancer Effect Levels (CEL) in Table 2-2 and are plotted in Figure 2-2.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans following dermal exposure to 4,4′-methylenedianiline. Very limited information exists regarding death in animals after dermal exposure to 4,4′-methylenedianiline.

Four of 9 female mice and 1 of 9 males died after having doses of 168 mg 4,4′-methylenedianiline/kg/day in methanol applied to the clipped skin 5 days per week for 2 weeks (Holland et al. 1987). When the solvent was acetone, 3 of 10 males and 3 of 10 females died. The authors indicated that using acetone as solvent may have provided inaccurate results since 4,4′-methylenedianiline tends to form a Schiff base with acetone. The same group of investigators (Holland et al. 1987) reported a significant dose-related decrease in survival rate in mice applied ≥5.3 mg 4,4′-methylenedianiline/kg/day 3 times per week for 104 weeks. The 24-month survival rate was approximately 60%

in untreated controls and 35.9% in treated mice. LOAEL values for death for each species and duration category are recorded in Table 2-3.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory effects in humans or musculoskeletal effects in humans or animals after dermal exposure to 4,4′-methylenedianiline.

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

Respiratory Effects. A single study in animals indicates that no gross or histopathological lesions were detected in the lungs and trachea of rabbits that received daily skin doses of 12,000 mg 4,4′-methylenedianiline/kg as an aqueous paste for 10 consecutive days (DuPont 1975). No further relevant information was located.

Cardiovascular Effects. A case of a male subject with cardiac abnormalities after dermal exposure to 4,4′-methylenedianiline was described by Brooks et al. (1979). The 20-year-old subject had worked for 2 weeks at a chemical plant where he handled large quantities of 4,4′-methylenedianiline. Exposure had occurred mostly by dermal contact with uncovered portions of the arms six days prior to admission to the hospital. Analysis of the electrocardiogram on admission showed normal waves and tests for myocardial damage were unremarkable. However, results from an echocardiogram showed reduced septal motion and reduced left ventricular function. Myocardial abnormalities were still observed three months after exposure, but not one year after the incident. No information was provided regarding possible simultaneous exposure to other chemicals.

No studies were located regarding cardiovascular effects in animals after dermal exposure to 4,4′-methylenedianiline.

Gastrointestinal Effects. No studies were located regarding, gastrointestinal effects in humans after dermal exposure to 4,4′-methylenedianiline.

Table 2-3. Levels of Significant Exposure to 4,4'-Methylenedianiline - Dermal

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
ACUTE EXPOSURE						
Death						
Mouse (C3Hf/Bd)	2 wk 5 d/wk				168 F (4/9 died)	Holland et al. 1987
Systemic						
Mouse (C3Hf/Bd)	2 wk 5 d/wk	Hepatic	84	168 (increased absolute liver weight)		Holland et al. 1987
		Renal	168			
		Dermal	168			
		Bd Wt	168			
Gn Pig (Albino Hartley)	once	Dermal	22M			Leong et al. 1987
Rabbit (Albino)	10 d 6 x/d	Hepatic			700 M (bile duct proliferation, portal cirrhosis, focal parenchymal necrosis in liver)	DuPont 1976a
		Renal		700 M (mild acute glomerulonephritis)		
		Dermal			700 M (acute necrotizing dermatitis)	
		Bd Wt		700 M (15% reduction in final body weight)		
Rabbit (NS)	once	Ocular		3.3 (reversible corneal opacity; conjunctivitis; iris congestion)		DuPont 1976b

Table 2-3. Levels of Significant Exposure to 4,4'-Methylenedianiline - Dermal (continued)

Species (Strain)	Exposure/ duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
CHRONIC EXPOSURE						
Death						
Mouse (C3Hf/Bd)	104 wk 3 d/wk				5.3 F (15.4% survival rate; 60% in controls)	Holland et al. 1987
Cancer						
Mouse (C3Hf/Bd)	104 wk 3 d/wk				5.3 F (CEL-increased incidence of hepatic tumors)	Holland et al. 1987

Bd Wt = body weight; CEL = cancer effect level; d = day(s); Derm = dermal; F = female; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed- adverse-effect level; NS = not specified; wk = week(s); x = time(s)

A single study in animals indicate that no gross or histopathological lesions were detected in the stomach and intestines of rabbits that received daily skin doses of up to 2,000 mg 4,4′-methylenedianiline/kg as an aqueous paste for 10 consecutive days (DuPont 1975). No further relevant information was located.

Hematological Effects. Very limited information was located regarding hematological effects in humans following dermal exposure to 4,4′-methylenedianiline. Normal blood counts were reported in a man following accidental contact with 4,4′-methylenedianiline (Van Joost et al. 1987). A second case concerned six men who came in contact with 4,4′-methylenedianiline while mixing it with an epoxy resin at work (Williams et al. 1974). Four of the six men were reported to have elevated eosinophil count, which is consistent with an immunological allergic reaction (although none was described) (see Section 2.5).

No studies were located regarding hematological effects in animals after dermal exposure to 4,4′-methylenedianiline.

Hepatic Effects. Several studies have described adverse hepatic effects in humans after dermal exposure to 4,4′-methylenedianiline. Thirteen cases of toxic hepatitis were reported in a factory that manufactured hard plastic (McGill and Motto 1974). 4,4′-Methylenedianiline was used as a curing agent in the process. The illness began between one and three weeks after employment started and all the reported signs and symptoms were consistent with liver disease (right upper quadrant pain and fever, jaundice, elevated transaminases, hyperbilirubinemia). Similar findings have also been described by others (Bastian 1984; Brooks et al. 1979; Williams et al. 1974). All these cases shared common signs and symptoms that included pain, elevated serum transaminases, jaundice, and hyperbilirubinemia. Although simultaneous exposure to other chemicals cannot be totally ruled out, the overall evidence and results from animal studies suggest that 4,4′-methylenedianiline was a major contributor to liver toxicity.

Limited data from animal studies suggest that the liver may also be a target for 4,4′-methylenedianiline after dermal exposure. Mice that received daily applications of 168 mg 4,4′-methylenedianilinelkg in methanol or acetone 5 days per week for 2 weeks exhibited an increase in liver relative to vehicle controls (Holland et al. 1987). No effects were seen at 84 mg/kg/day. Bile duct proliferation, portal cirrhosis, and focal parenchymal necrosis were observed in the livers of rabbits

which received skin doses of 700 mg 4,4′-methylenedianiline/kg/day in ethanol for 10 consecutive days (DuPont 1976a). These changes were not noticed in rabbits treated with ethanol alone. In contrast, no adverse hepatic effects were noticed in the livers of rabbits also treated for 10 consecutive days with up to 2,000 mg 4,4′-methylenedianiline/kg/day, but applied as an aqueous paste (DuPont 1975), suggesting that the vehicle plays an important role in dermal absorption. No further information was located regarding hepatic effects after dermal exposure.

Renal Effects. No studies were located regarding renal effects in humans after dermal exposure to 4,4′-methylenedianiline.

No adverse kidney effects were reported in mice which received up to 168 mg 4,4′-methylenedianiline/kg/day in methanol or acetone applied 5 days per week for 2 weeks (Holland et al. 1987).
Mild acute glomerulonephritis was reported in rabbits treated with 700 mg 4,4′-methylenedianiline/kg/day in ethanol for 10 consecutive days (DuPont 1976a). However, no such effect was noticed when 2,000 mg 4,4′-methylenedianiline/kg/day was applied as an aqueous paste (DuPont 1975), indicating that the vehicle plays a role in dermal absorption. No further information was located regarding renal effects after dermal exposure.

Endocrine Effects. No studies were located regarding endocrine effects in humans after dermal exposure to 4,4′-methylenedianiline.

The only information regarding endocrine effects in animals is that provided in a study in which no gross or histopathological alterations were seen in the adrenals and thyroid of rabbits which received doses of to 2,000 mg 4,4′-methylenedianiline/kg/day as an aqueous paste to the skin for 10 consecutive days (DuPont 1975).

Dermal Effects. Skin rash was one of the physical findings among a group of 13 individuals who came in contact with 4,4′-methylenedianiline at work (McGill and Motto 1974). However, since there was clinical evidence of liver disease, the rash may have been another sign of toxic hepatitis and not due to direct contact with the chemical. A similar clinical picture was described by Brooks et al. (1979) in a male who handled large amounts of 4,4′-methylenedianiline at a chemical plant. Dermatitis without evidence of liver damage has also been reported (Emmett 1976; Van Joost et al. 1987). It appeared that in these cases dermal sensitization had occurred since patch testing with

4,4′-methylenedianiline gave positive reactions. A case of photosensitivity to 4,4′-methylenedianiline was reported in a male subject who developed erythematous, pruritic dermatitis on his arms and forearms during four consecutive summers (Levine 1983). The rash appeared 60 minutes after a 30 minute exposure to sunlight even if filtered by window glass. Photopatch tests conducted with 24 contact allergens were positive for 4,4′-methylenedianiline.

No dermal irritation at the application site was observed in mice treated with up to 168 mg 4,4′-methylenedianiline/kg/day in ethanol or acetone 5 days per week for 2 weeks (Holland et al. 1987). Ten daily doses of 700 mg 4,4′-methylenedianiline/kg in ethanol produced acute necrotizing dermatitis in rabbits (DuPont 1976a). However, when the test material was applied as an aqueous paste, ten doses of 1,000 mg 4,4′-methylenedianiline/kg produced only minimal irritation (DuPont 1975). Application of a single 22 mg 4,4′-methylenedianilinelkg in polyethylene glycol to the back of guinea pigs produced neither dermal irritation nor sensitization (Leong et al. 1987). In this study, the guinea pigs had been previously exposed to an aerosol of 4,4′-methylenedianiline intermittently for 2 weeks in order to determine whether dermal sensitization to 4,4′-methylenedianiline occurred across routes of exposure.

Ocular Effects. No studies were located regarding ocular effects in humans after dermal exposure to 4,4′-methylenedianiline.

No gross or histopathological lesions were observed in the eyes of rabbits after receiving daily skin applications of up to 2,000 mg 4,4′-methylenedianiline/kg as an aqueous paste for 10 consecutive days (DuPont 1975). Moderate to mild reversible ocular effects were seen in the eyes of rabbits after solid 4,4′-methylenedianiline (3.3 or 33.3 mg/kg) was placed into the conjunctival sac for 20 seconds (DuPont 1976b). Effects observed included cornea1 opacity, congestion of the iris, and redness and swelling of the conjunctiva. The severity of the effects was dose-related and washing with water for 3.5 minutes after the 20-second treatment considerably lessened the severity.

Body Weight Effects. No studies were located regarding body weight effects in humans following dermal exposure to 4,4′-methylenedianiline.

No significant alterations in body weight were observed in mice treated 5 days per week for 2 weeks with up to 168 mg 4,4′-methylenedianiline/kg/day in acetone or ethanol (Holland et al. 1987). Similar

results were reported in rabbits treated with up to 2,000 mg 4,4′-methylenedianiline/kg as an aqueous paste for 10 consecutive days (DuPont 1975). However, when the solvent was ethanol, there was a 15% reduction in final body weight after 10 days of treatment with 700 mg 4,4′-methylenedianiline/ kg/day, which suggests that ethanol facilitates dermal absorption of this chemical (DuPont 1976a).

2.2.3.3 Immunological and Lymphoreticular Effects

Several cases of dermal sensitization have been described in individuals who came in contact with 4,4′-methylenedianiline in the workplace (Emmett 1976; Levine 1983; Van Joost et al. 1987) (see Dermal Effects). However, no information is available regarding possible effects of 4,4′-methylenedianiline on human immunocompetence.

No dermal sensitization was observed in guinea pigs after a 2-week nose-only exposure period to 4,4′-methylenedianiline aerosol was followed with a single topical application of up to 22 mg 4,4′-methylenedianiline/kg (Leong et al. 1987). Increased spleen weight was reported in mice that received topical applications of up to 168 mg 4,4′-methylenedianiline/kg/day in ethanol for 2 weeks (Holland et al. 1987); no further information was provided in that report. No gross or histopathological alterations were observed in the spleen and thymus of rabbits treated dermally with 2,000 mg 4,4′-methylenedianiline/kg as an aqueous paste for 10 consecutive days (DuPont 1975). The information available is insufficient to draw any conclusions regarding immunological effects of 4,4′-methylenedianiline after dermal exposure and, therefore, no entries are presented in Table 2-3.

2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans after dermal exposure to 4,4′-methylenedianiline.

The information regarding neurological effects in animals is limited to a study that reported no treatment-related gross or histopathological alterations in the brains of rabbits after receiving 10 consecutive daily skin applications of up to 2,000 mg 4,4′-methylenedianiline/kg as an aqueous paste (DuPont 1975). No further neurological parameters were examined. This brief information is not considered a reliable indicator for neurological effect and, therefore, is not listed in Table 2-3.

2.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following dermal exposure to 4,4′-methylenedianiline.

The only information regarding reproductive effects in animals is that provided in a study in which no gross or histopathological alterations were seen in the testes and epididymis of rabbits which received up to 2,000 mg 4,4′-methylenedianiline/kg/day applied as an aqueous paste to the skin for 10 consecutive days (DuPont 1975). No other reproductive parameters were evaluated. Because this information is not considered a reliable indicator of reproductive function, it is not listed in Table 2-3.

2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to 4,4′-methylenedianiline.

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after dermal exposure to 4,4′-methylenedianiline. Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

A retrospective assessment of exposure and cancer morbidity was conducted in power generation workers exposed to an epoxy resin containing 35% 4,4′-methylenedianiline in Sweden (Selden et al. 1992). The cohort was composed of 550 males and 45 females. Based on company records, the individuals were subdivided into three categories: exposed, possibly exposed, and unexposed. Information on the cancer incidence of the cohort was obtained by computerized matching with the national cancer register for the period 1964-1985. Standardized incidence ratios (SIR) were obtained from the ratio of the observed to the expected number of cases. Exposure was considered to be primarily by the dermal route. In all three male groups, the observed number of cancers for all sites and for urinary bladder cancer was lower than the observed number. The overall SIR was 0.52 based on 5 observed cases. In the male exposed subgroup, no single cancer case appeared throughout the

observation period; the expected number was 3. Among the female workers, 2 cancer cases were identified (none in the urinary bladder); 2.7 cancer cases (all cancers) had been expected from the national rates. The authors indicate that the results should be interpreted with caution since the cohort was small, the majority of the subjects were quite young and had not reached cancer-prone age, and the follow-up period was short and may have not covered the latency period for bladder cancer (20 years).

Additional information is available from a morbidity study of employees who had worked in the gas centrifuge process at Oak Ridge Gaseous Diffusion Plant (Cragle et al. 1992). In addition to potential exposure to 4,4′-methylenedianiline, the workers may have been exposed to m-phenylenediamine, bis(2,3-epoxycyclopentylether), diglycidyl ether of bisphenol A, and the solvents trichloroethylene and methylene chloride. The cohort consisted of 263 workers who had worked closest to the process for the longest amount of time and a comparison group of 271 workers who did not work in the process. The most significant finding of the study was the report of five bladder cancers among centrifuge workers and none among the comparison worker group. However, interviews with these five workers revealed that none of them was working closely with the epoxy resin materials during any part of their employment in the process. The authors concluded that no specific agent or job duty could be identified as the causative factors for the bladder cancers (Cragle et al. 1992).

A more recent study followed a group of 10 individuals who had worked at a plant in Ontario, Canada, that manufactured an epoxy concrete surfacing material using 4,4′-methylenedianiline as a hardener (Liss and Guirguis 1994). Between 1967 and 1976 these subjects suffered acute episodes of jaundice. The study followed the group from the date of intoxication through the end of 1991 for cancer incidence by matching with the Ontario Cancer Registry. At the time of the intoxication the length of employment ranged from 7 days to 2.5 months. The results of the follow-up revealed that one case of bladder cancer developed among the group; it was diagnosed in 1990, 23 years after the intoxication. The standardized incidence ratio for the bladder cancer was 19.3 (95% confidence interval 0.5-107; p [one or more cases]=0.051). Liss and Guirguis (1994) suggested that their results should be interpreted with caution, given the small number of events, the absence of smoking histories, and the presence of other exposures.

The potential carcinogenicity of 4,4′-methylenedianiline by the dermal route was examined in mice (Holland et al. 1987). A solution of the test material in ethanol was applied to the clipped skin of

male and female C3Hf/Bd mice 3 times per week for 24 months. Positive controls were treated with benzo[a]pyrene and negative controls with vehicle alone. Estimated doses were 5.3, 10.7, and 21.3 mg 4,4′-methylenedianiline/kg/day. Treatment with 4,4′-methylenedianiline did not produce tumors at the application site, but increased the incidence of hepatic tumors in females in a dose-related manner (11% vehicle control, 22% low-dose, 25% mid-dose, 85% high-dose); however, a statistical analysis of the results was not provided. The incidence of tumors in the spleen, lungs, kidneys, and ovaries/testes did not increase relative to negative controls. According to the investigators, the C3Hf/Bd strain of mice is unusually susceptible to liver tumors and, therefore, the significance of the findings requires further study. Nevertheless, the dose of 5.3 mg 4,4′-methylenedianiline/kg/day is listed as a Cancer Effect Level in Table 2-3.

In summary, there is insufficient information to assess the potential carcinogenicity of 4,4′-methylenedianiline by the dermal route of exposure.

2. 3 TOXICOKINETICS

Data regarding toxicokinetics of 4,4′-methylenedianiline in humans are limited to information from cases of accidental ingestion of food contaminated with the chemical and cases of occupational exposure in the workplace, where dermal contact with 4,4′-methylenedianiline is considered the predominant route of exposure. Humans can absorb 4,4′-methylenedianiline by the inhalation, oral, and dermal routes of exposure. Limited data suggest that in humans the rate of absorption of 4,4′-methylenedianiline through the respiratory tract is faster than dermal absorption. There are no data regarding quantitative oral absorption in humans or animals. There are no inhalation data in animals. Limited dermal data in animals showed a higher absorption rate in rats than in guinea pigs. Furthermore, the absorption rate was a saturable process. No remarkable pattern of accumulation of 4,4′-methylenedianiline occurred in tissues of either species, although the liver seemed to have a higher concentration of 4,4′-methylenedianiline than other tissues. 4,4′-Methylenedianiline may be metabolized by cytochrome P-450 to polar metabolites that can undergo conjugation with glutathione; this is inferred from data on structurally similar chemicals. 4,4′-Methylenedianiline can also be acetylated and, in humans and animals, such metabolites have been detected in the urine. Because of the limited data available, a physiologically based pharmacokinetic (PBPK) model for 4,4′-methylenedianiline has not been developed.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Only qualitative information is available regarding absorption of 4,4′-methylenedianiline by the inhalation route in humans. Biological monitoring was used to assess exposure to 4,4′-methylenedianiline in a cohort composed of 411 men employed in industries that manufactured or used 4,4′-methylenedianiline in the United Kingdom (Cocker et al. 1994). The results showed that when exposure to 4,4′-methylenedianiline was through inhalation, postshift urine samples had higher concentration of 4,4′-methylenedianiline than samples taken preshift the next day. This suggested that 4,4′-methylenedianiline is rapidly absorbed through the inhalation route and that peak excretion is reached after the end of the shift. In a recent study (Schiitze et al. 1995), 4,4′-methylenedianiline was found in the urine of 33 workers exposed to low levels of 4,4′-methylenedianiline. By using personal air samplers to monitor exposure, the authors found that most workers were exposed to <20 μg 4,4′-methylenedianiline/m3, the detection limit. The metabolite of 4,4′-methylenedianiline, N'-acetylmethylenedianiline, was found in the urine of all but 4 workers. In addition, hemoglobin adducts of 4,4′-methylenedianiline were found in all blood samples from the exposed workers. The possibility of dermal absorption was not discussed.

Very limited, and only indirect evidence of pulmonary absorption of 4,4′-methylenedianiline exists in animals. Guinea pigs exposed nose-only to an aerosol of 4,4′-methylenedianiline intermittently for 2 weeks showed retinal lesions, which were attributed to exposure to the test compound (Leong et al. 1987). This indicates that absorption through the respiratory tract had occurred. No further information was located.

2.3.1.2 Oral Exposure

In 1965, a group of 84 subjects in Epping, England, developed clinical signs consistent with toxic hepatitis shortly after eating bread prepared with flour that was later found to have been contaminated with 4,4′-methylenedianiline (Kopelman et al. 1966). This was the first documented case of human poisoning with 4,4′-methylenedianiline and provided clear evidence that the chemical can be absorbed from the gastrointestinal tract. No quantitative estimates of absorption were made. A later case report described a variety of toxic effects in a man who accidentally ingested a solution containing

4,4′-methylenedianiline, potassium carbonate, and gamma-butyrolactone (Roy et al. 1985). Although in this case there was simultaneous ingestion of other chemicals, some clinical signs were consistent with 4,4′-methylenedianiline toxicity and provide further evidence of oral absorption in humans.

Oral absorption of 4,4′-methylenedianiline in animals can be inferred from the numerous reports of toxic effects after oral administration of 4,4′-methylenedianiline summarized in Section 2.2.2. Gastrointestinal absorption occurred when 4,4′-methylenedianiline was administered by gavage, mixed with food, or in the drinking water. However, no quantitative data exist.

Further evidence of oral absorption is provided in a study in which metabolites of 4,4′-methylenedianiline were detected in the urine from rats administered a single dose of the test material (Tanaka et al. 1985). The extent of absorption was not determined.

2.3.1.3 Dermal Exposure

Indirect evidence of dermal absorption of 4,4′-methylenedianiline in humans is provided by the various reports of adverse health effects observed in individuals exposed to the chemical in the workplace; these studies are summarized in Section 2.2.3. It should be mentioned, however, that in some of these cases inhalation of dusts of 4,4′-methylenedianiline cannot be ruled out. Evidence of dermal absorption was also presented by Cocker et al. (1986a, 1994) who detected 4,4′-methylenedianiline and/or metabolites in the urine of workers exposed primarily by the dermal route. Quantitative data were not provided in these studies. A recent study by Bnmmark et al. (1995) showed that approximately 28% of a dose of 4,4′-methylenedianiline in isopropanol applied in a patch for 1 hour to the ventral skin of the forearm of male volunteers was absorbed. In that study, 4,4′-methylenedianiline reached a peak in hydrolyzed plasma 3-4 hours after initiation of exposure and declined thereafter, with a time course consistent with first-order, one compartment kinetics. Additional information can be drawn from a study that used human skin *in vitro* (Hotchkiss et al. 1993). When the skin was unoccluded after application of 4,4′-methylenedianiline in ethanol, 13% of -the applied dose was detected in a receptor fluid at 72 hours. When the skin was occluded, 33% of the applied dose appeared in the receptor fluid at 72 hours. It was also observed that a considerable amount of 4,4′-methylenedianiline (23-58% of the applied dose) remained within the skin at the end of the experiment. The authors noted that this finding may be of concern in terms of exposure in the

workplace, where gloves or clothing may be worn on top of the chemicals after they come in contact with the skin.

Quantitative aspects of dermal absorption have been examined in rats, guinea pigs and monkeys. A single dose of ^{14}C-ring labeled 4,4′-methylenedianiline (2 or 20 mg/kg) in ethanol/water was applied to the back of rats and the area was covered with a cup (El-Hawari et al. 1986). After an exposure of 6 hours, a total of approximately 12% of the applied radioactivity was recovered in the urine, feces, gastrointestinal tract and tissues; 62% was recovered in a wash with soap and water, and 30% remained in the application site (total recoveries from radiotracer studies often exceed 100%). A 24-hour exposure period resulted in a combined 27% of the dose in urine, feces, gastrointestinal tract and tissues, 52% in the wash, and 25% in the application site. After a 96-hour exposure period, 55% of the dose was accounted for by the combined urine, feces, gastrointestinal tract, and tissues, 25% was in the wash and 26% in the application site. The results also showed that after washing, test material which remained within the skin continued to be absorbed. Moreover, occlusion facilitated absorption. When two dose levels were tested, the amount of radioactivity in tissues was higher after the high dose, but the proportion of the applied dose was lower than with the low dose, which suggested a dose-dependent absorption rate. Finally, a greater percentage of the dose was absorbed when the application site was washed with acetone and water 5 minutes after dosing than when washed with soap and water.

In guinea pigs subjected to the same protocol (El-Hawaii et al. 1986), approximately 3.5% of the applied dose (2 or 20 mg/kg) was recovered in the urine, feces, gastrointestinal tract and tissues immediately after a 6-hour exposure period; 81% was recovered in the wash and 11% remained in the application site. After a 96-hour exposure period, recovery in urine, feces, gastrointestinal tract and tissues amounted to 30% of the dose. Occlusion of the exposure site did not significantly affect absorption rate. In contrast with the results in rats, washing the area with soap and water prevented further absorption. On the other hand, as seen in rats, absorption rate was dose-dependent and a wash with acetone and water facilitated absorption relative to washing with soap and water.

A less comprehensive study was conducted in monkeys (El-Hawari et al. 1986). The treatment protocol was similar to that used in rats and guinea pigs, but the dose was kept in place for only 24 hours, after which time the site was washed with soap and water. As a percentage of the applied dose, the cumulative excretion of radioactivity over a 168-hour period was 18.8% in the urine and

1.9% in feces. Forty-seven percent was recovered in the wash for a total recovery of about 68 % Since tissues from monkeys were not analyzed, a 21% absorption rate represents only a lower limit. When the application site was washed with soap and water 5-10 minutes after the 24-hour exposure period, 63% was recovered in the wash; when acetone and water were used, recovery was 53%.

A study with rat skin *in vitro* showed that after application of 4,4′-methylenedianiline in ethanol to an unoccluded piece of skin, about 6% of the applied dose reached a receptor compartment at 72 hours (Hotchkiss et al. 1993). When the application site was occluded, absorption was enhanced, reaching 13.3% of the applied dose in 72 hours. These values are lower than those observed in experiments with human skin *in vitro* (Hotchkiss et al. 1993).

Dermal absorption has not been quantitated in mice, but toxic effects observed after dermal exposure, reported in studies summarized in Section 2.2.3, indicate that 4,4′-methylenedianiline is absorbed in this species.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution of 4,4′-methylenedianiline in humans following inhalation exposure.

From the study by Leong et al. (1987) (Section 2.2.1) in which retinal toxicity was reported in guinea pigs exposed nose-only to an aerosol of 4,4′-methylenedianiline it could be inferred that 4,4′-methylenedianiline (or metabolites) reached the eye after pulmonary absorption. No further relevant information was located.

2.3.2.2 Oral Exposure

The fact that subjects who ate bread made with flour contaminated with 4,4′-methylenedianiline developed toxic hepatitis (Kopelman et al. 1966) provides indirect evidence that in humans, 4,4′-methylenedianiline distributes to the liver after oral exposure. In another study (Roy et al. 1985), a subject who accidentally drank a solution containing 4,4′-methylenedianiline, potassium carbonate,

and gamma-butyrolactone showed adverse effects on the liver and kidneys, and also had severe loss of visual acuity (Section 2.2.2). This could indicate that 4,4′-methylenedianiline (or metabolites) distribute to the liver, kidney and eye. However, because other chemicals were involved, the evidence is inconclusive.

Studies regarding quantitative distribution of 4,4′-methylenedianiline in tissues and organs from animals after oral exposure were not located. Nevertheless, the numerous reports that showed toxic responses in various organs after oral administration of 4,4′-methylenedianiline (summarized in Section 2.2.2) indicate that 4,4′-methylenedianiline (or metabolites) can distribute to these organs. The organs involved are the liver, kidneys, spleen, thymus, uterus, adrenal glands, and thyroid.

2.3.2.3 Dermal Exposure

Case reports of humans who developed hepatitis after dermal contact with 4,4′-methylenedianiline in the workplace provide indirect evidence that 4,4′-methylenedianiline distributes to the liver. No further information was located regarding distribution in humans after dermal exposure.

Quantitative distribution studies have been conducted in rats and guinea pigs after skin application of ^{14}C-ring-labeled 4,4′-methylenedianiline (El-Hawari et al. 1986). Rats were applied a single dose (2 and 20 mg/kg) of the test material in ethanol/water and the dose area was covered with a cup. The dose was kept on site for 6, 24, or 96 hours. After these times, the skin was washed with soap and water and the animals were either sacrificed or returned to the cages for later sacrifice. Organs and tissues were prepared for radiochemical analysis. The results showed that the gastrointestinal tract contained the highest amounts of radioactivity at 6 (3.8%) and 24 (3%) hours, followed by the liver (2% and 1.2%, respectively). After 96 hours, both the gastrointestinal tract and liver had about 0.5% of the applied radioactivity. On a per gram basis, the liver had the highest amount of radioactivity at all times, followed by the adrenals and kidneys; this was also the case with the high dose. With the exception of the liver, preferential accumulation of 4,4′-methylenedianiline (or metabolites) was not apparent.

The experimental procedure and dose levels in guinea pigs were the same as in the rat study (El-Hawari et al. 1986). The gastrointestinal tract had the highest amount of radioactivity at 6 (1.5%) and 24 (2.8%) hours followed by the liver (0.4% and 0.5 %, respectively). After 96 hours, both the

gastrointestinal tract and liver contained about 0.5% of the applied dose. On a per gram basis, the adrenals had the highest amount of radioactivity at all times (about 3 times the liver). The distribution pattern did not appear to be dose-dependent and preferential accumulation in organs and tissues was not apparent.

2.3.2.4 Other Routes of Exposure

The distribution of 4,4′-methylenedianiline (or metabolites) has also been studied in rats and guinea pigs after a single intravenous injection (El-Hawari et al. 1986). Rats were injected ^{14}C-ring-labeled 4,4′-methylenedianiline (2 mg/kg) in ethanol water and sacrifices were conducted 6, 24, or 96 after dosing. After 6 hours, the gastrointestinal tract had the highest amount of radioactivity (24% of the dose); this was followed by the liver (9.5%), skin (3.2%), and blood (2.8%). Twenty-four hours after the injection, the amount of radioactivity had decreased considerably in all tissues, and the liver and gastrointestinal tract had about 4% of the administered dose. Ninety-six hours after dosing, the liver had 4 or more times higher radioactivity (0.9%) than any other tissue. On a per gram basis, the liver had the highest concentration of radioactivity at all times, followed by the lungs at 6 and 24 hours and the spleen at 96 hours. Except for the liver, no preferential accumulation was apparent.

The guinea pigs were treated the same as the rats except that sacrifices were conducted only 96 hours after the injection (El-Hawari et al. 1986). As a percentage of the applied dose, the liver had the most radioactivity, about 3 times that found in blood. On a per gram basis, radioactivity was most concentrated in the spleen, followed by the liver, and preferential accumulation in these organs was suggested. Total recovery as a percentage of the dose, in blood, tissues, and gastrointestinal tract was 0.55%, 2.4% and 0.61%, respectively.

2.3.3 Metabolism

Limited information exists regarding the metabolism of 4,4′-methylenedianiline. By inference from structurally similar compounds, it has been assumed that 4,4′-methylenedianiline is oxidized to N-hydroxymethylenedianiline by the monooxygenase system (Cocker et al. 1986a; Farmer and Bailey 1989). This reaction leads to the formation of potentially toxic derivatives that may bind to cell macromolecule. The aryl hydroxylamine can be further oxidized to nitrosomethylenedianiline which can then be conjugated with glutathione and excreted in the urine. A different type of reaction is

acetylation of 4,4′-methylenedianiline to form N-acetylmethylenedianiline and N,N' diacetylmethyl-enedianiline. Both the mono- and di-acetylated metabolites have been identified in the urine of exposed workers (Robert et al. 1995). N-acetylmethylenedianiline has been identified in the urine from rats treated with 4,4′-methylenedianiline orally (Tanaka et al. 1985) and in the urine of humans exposed to the chemical in the workplace (Cocker et al. 1986a, 1994; Schtitze et al. 1995). This apparently represents a detoxification pathway since acetylated metabolites are not mutagenic (Cocker et al. 1986b; Tanaka et al. 1985). Results from an *in vitro* study in which 4,4′-methylenedianiline was incubated with rabbit liver microsomes showed that three metabolites were formed: azodiphenylmethane, azoxydiphenylmethane, and 4-nitroso-4'-aminodiphenylmethane (Kajbaf et al. 1992). According to the investigators, the latter may have been formed via a nonenzymatic reaction, whereas the former two were produced enzymatically. A schematic diagram of the metabolism of 4,4′-methylenedianiline is presented in Figure 2-3.

2.3.4 Elimination and Excretion

2.3.4.1 Inhalation Exposure

Data in humans are provided by a study which detected 4,4′-methylenedianiline (cl00 nmol/mmol creatinine) in the urine of workers exposed through inhalation of solid material or contaminated dust (Cocker et al. 1994). A recent study found levels of 4,4′-methylenedianiline from 0.013 to 2.76 nmol/L in the urine of workers exposed presumably by inhalation to low levels of 4,4′-methylenedianiline (Schtitze et al. 1995). The urinary concentration of the metabolite N'-acetylmethylenedianiline ranged from 0.045 to 23.4 nmol/L. No further information was located.

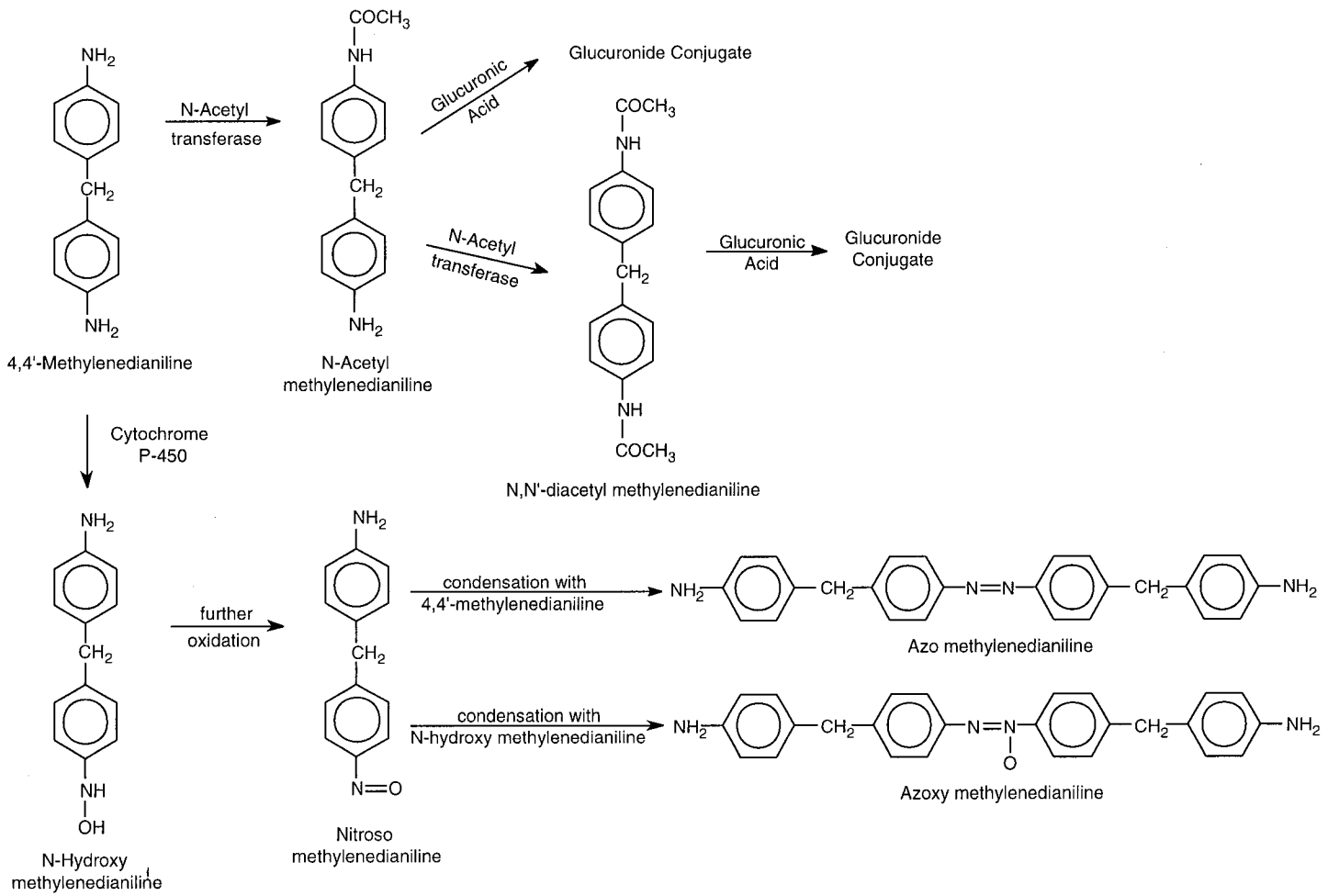
No studies were located regarding elimination and excretion of 4,4′-methylenedianiline or metabolites in animals after inhalation exposure.

2.3.4.2 Oral Exposure

No studies were located regarding elimination and excretion of 4,4′-methylenedianiline ormetabolites in humans after oral exposure.

Information regarding animals is limited to a report by Tanaka et al. (1985), who demonstrated the presence of the N-acetyl and N,N'-diacetyl derivatives of 4,4′-methylenedianiline in the urine of rats

Figure 2-3. Proposed Metabolic Pathway for 4,4′-Methylenedianiline



Source: Adapted from Kajbaf et al. 1992; Cocker et al. 1986a, 1994

treated with a single gavage dose of 50 mg/kg of the test material in gum arabic. No quantitative information was provided.

2.3.4.3 Dermal Exposure

4,4′-Methylenedianiline and an N-acetyl conjugate of methylenedianiline have been identified in the urine of individuals who had dermal contact with 4,4′-methylenedianiline in the workplace (Cocker et al. 1986a, 1994). Over 300 urine samples were analyzed and in 81% the concentration of 4,4′-methylenedianiline was below the detection limit for the method (gas chromatography-mass spectrometry) (Cocker et al. 1986a). In those found to contain 4,4′-methylenedianiline, the concentration ranged from 6 to 175 nmol/mmol creatinine; the average was 26 nmol/mmol creatinine. N,N'-diacetylmethylenedianiline has also been found in the urine of exposed workers, although at much lower concentrations than the monoacetyl derivative (Robert et al. 1995). A recent study examined the excretion of 4,4′-methylenedianiline in seven male workers from a work site where the chemical was used as curing agent for an epoxy resin (Dalene et al. 1995). The study was conducted during 4 workdays and one weekend. Exposure was considered to be mainly dermal in spite of extensive protection measures. The excretion rate in urine ranged from 0 to 90 μmol/hour and was lower after the weekend than after workdays. On workdays, the cumulative excretion ranged from 0.04 to 1.2 μmol/day; during the weekend, the range was 0.005-0.51 μmol/day. The excretion of 4,4′-methylenedianiline in the urine correlated linearly with its concentration in blood. 4,4′-Methylenedianiline was applied to 5 volunteers in a patch to the ventral forearm for 1 hour; excretion of the compound in hydrolyzed urine reached a peak 6-l1 hours after exposure and most of the 4,4′-methylenedianiline was eliminated within 24 hours (Brunmark et al. 1995). Of the dose absorbed, a median of 16% was excreted in the urine. Half-life elimination ranged from 9.2 to 19 hours in plasma and from 4.6 to 11 hours in urine. In two volunteers who received three dose levels, elimination half-lives were not dose-dependent (Brunmark et al. 1995).

Quantitative information regarding excretion of 4,4′-methylenedianiline is available from studies in rats, guinea pigs, and monkeys after skin application of ^{14}C-ring-labeled 4,4′-methylenedianiline (El-Hawari et al. 1986). Rats were applied a single dose (2 or 20 mg/kg) of the test material in ethanol/water and the dose area was covered with a cup. The dose was kept on site for 6, 24, or 96 hours. After these times, the skin was washed with soap and water and the animals were either sacrificed or returned to the cages for later sacrifice. Urine and feces were collected at various

intervals for up to 96 hours. After a 6-hour exposure, 2.5% and 0.04% of the administered radioactivity were recovered in urine and feces, respectively. After a 24-hour exposure, urine and feces had 20% and 2.3% of the dose, respectively. The corresponding values for a 96-hour exposure were 43 % and 10%. Rats in which the application site was not covered had lower amounts of radioactivity in the excreta. The distribution of radioactivity between urine and feces was not dose dependent. The results showed that the main excretory route in the rat is the urine.

In guinea pigs subjected to the same protocol (El-Hawari et al. 1986), 0.35% and 0.1% of the administered dose were recovered in urine and feces, respectively. After a 24-hour exposure, the recoveries in urine and in feces were 7.8% and 5.7%, respectively. The corresponding values for a 96-hour exposure were 10.5% and 17.6%. Treatment with the high dose also resulted in similar percentages of radioactivity in urine (2.8%) and feces (3.6%). In contrast with results in the rat, both urine and feces appear to be main excretory routes in guinea pigs.

In monkeys, the treatment protocol was similar to that used in rats and guinea pigs, but the dose was kept in place for only 24 hours, after which time the site was washed with soap and water (El-Hawari et al. 1986). Following dosing, urine and feces were collected for up to 168 hours. As a percentage of the applied dose, the cumulative excretion of radioactivity in the urine and feces over the 168-hour period was 18.8% and 1.9%, respectively, indicating that in monkeys, the urine is the main excretory route for 4,4′-methylenedianiline or metabolite(s).

2.3.4.4 Other Routes of Exposure

The pattern of excretion of 4,4′-methylenedianiline or metabolites was also examined in rats, guinea pigs, and monkeys after a single intravenous dose of 2 mg/kg of ^{14}C-ring-labeled 4,4′-methylenedianiline (El-Hawari et al. 1986). In rats and guinea pigs, urine and feces were collected for up to 96 hours after dosing; in monkeys, excreta were collected for 168 hours. In rats, the amount of radioactivity recovered in the urine and feces 6 hours after the injection was (as a percentage of the dose) 55% and 0.3%, respectively. After 24 hours, the respective percentages were 67.4% and 21.8% and, after 96 hours, 67 % and 31%. This indicates that the urine is the main excretory route and that excretion was almost complete 24 hours after the injection.

In guinea pigs, urinary and fecal excretion reached maximums of 34% and 5 1% of the dose, respectively, at about 48 hours after dosing, thus indicating that the feces is the main route of elimination of 4,4´-methylenedianiline in guinea pigs (El-Hawari et al. 1986).

In monkeys, most of the dose (79%) was excreted in the urine within the first 48 hours; at this time 6.5% of the dose appeared in the feces (El-Hawari et al. 1986). Total recovery of radioactivity over a the 168-hour collection period amounted to 94% of the injected dose. As seen in rats, urine was the main route of excretion in monkeys.

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

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

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

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

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

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

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically-sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 2-4 shows a conceptualized representation of a PBPK model.

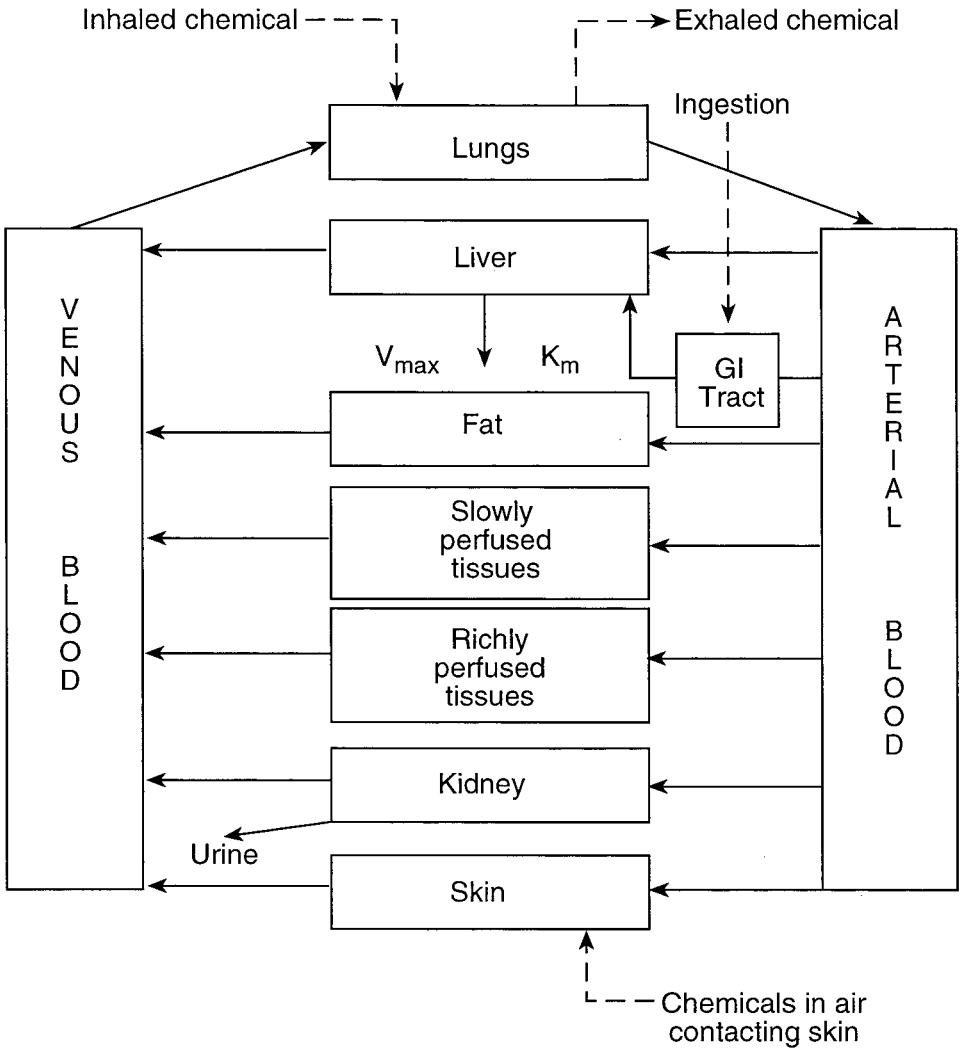
If PBPK models for 4,4′-methylenedianiline exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Very limited information exists regarding toxicokinetics of methylenedianiline; consequently, a PBPK model for 4,4′-methylenedianiline has not yet been developed.

2.4 MECHANISMS OF ACTION

The mechanism of 4,4′-methylenedianiline absorption in the gastrointestinal tract, lungs, or skin is not known. Also, no information is available on how 4,4′-methylenedianiline is transported in the blood

Figure 2-4. 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.

or stored in tissues. 4,4′-Methylenedianiline can undergo N-hydroxylation leading to the formation of intermediates suspected of being mutagenic and possibly carcinogenic. In contrast, N-acetylation, a reaction shown to occur in humans and animals, leads to less toxic derivatives. The liver and thyroid are targets for 4,4′-methylenedianiline toxicity in animals. Limited data indicate that the liver is also a target in humans. The existing information does not suggest route-specific toxicity.

2.4.1 Pharmacokinetic Mechanisms

The mechanism of absorption of 4,4′-methylenedianiline by the inhalation, oral, and dermal routes is not known. Based on the chemical properties of 4,4′-methylenedianiline (poorly soluble in water, soluble in lipids), a passive transfer process from the aqueous environment of the intestine across cell membranes can be anticipated. Limited dermal data in animals suggest that absorption by this route is a saturable process (El-Hawari et al. 1986). It was also shown in dermal studies that the administration vehicle plays a role in the extent of absorption. For example, 4,4′-methylenedianiline was more toxic to rabbits when applied in ethanol (DuPont 1976a) than when applied as an aqueous paste (DuPont 1975). The results from a study in humans exposed to 4,4′-methylenedianiline in the workplace suggested that pulmonary absorption is faster than dermal absorption (Cocker et al. 1994).

No information was located regarding the distribution of 4,4′-methylenedianiline in plasma, but it would be reasonable to assume that distribution is determined by partition among the various proteins according to lipid solubility and concentration. A first-pass effect in the liver can be expected. The limited toxicokinetics information available (El-Hawari et al. 1986) suggests that, with the exception of the liver, there is no preferential accumulation of 4,4′-methylenedianiline or metabolites in tissues or organs. No specific mechanism for storage of 4,4′-methylenedianiline or metabolites is apparent. Based on data from structurally-related compounds and from a study with 4,4′-methylenedianiline *in vitro* (Kajbaf et al. 1992), it seems that metabolism of 4,4′-methylenedianiline occurs in the liver. N-hydroxylation leads to N-hydroxylamine, a potentially toxic intermediate that can bind to macromolecule, or can be deactivated by conjugation with glutathione.

Humans and animals eliminate 4,4′-methylenedianiline in the urine (Cocker et al. 1986a, 1994; El-Hawaii et al. 1986; Tanaka et al. 1985). Results from a dermal study showed that rats and monkeys excrete 4,4′-methylenedianiline preferentially in the urine, whereas in guinea pigs both excretion routes are of equal importance (El-Hawari et al. 1986). The existing information on

4,4′-methylenedianiline does not suggest route-dependent toxicity, except for dermal effects which may occur after topical application of high concentrations of 4,4′-methylenedianiline.

2.4.2 Mechanisms of Toxicity

The mechanism of 4,4′-methylenedianiline toxicity is not completely understood. Based on information on structurally similar compounds, many of the toxic properties of 4,4′-methylenedianiline have been attributed to a reactive metabolic intermediate, N-hydroxymethylenedianiline, which results from the enzymatic oxidation of 4,4′-methylenedianiline (Cocker et al. 1986a; Lamb et al. 1986). In support of this view is the lack of genotoxicity of 4,4′-methylenedianiline in the absence of metabolic activation (Section 2.5). On the other hand, metabolic formation of N-acetylmethylenedianiline or N,N'-diacetylmethylenedianiline appears to represent a detoxification pathway since these metabolites were not mutagenic (Cocker et al. 1986b; Tanaka et al. 1985).

Results from studies summarized in Section 2.2 strongly s uggest that the liver is a target for 4,4′-methylenedianiline toxicity in humans and animals (Bailie et al. 1993, 1994; Hagiwara et al. 1993; Kanz et al. 1992; Kopelman et al. 1966; Schmidt et al. 1980) and that the thyroid may also be a target for toxicity in animals (Pukushima et al. 1981; Hiasa et al. 1984; NTP 1983; Tsuda et al. 1987). Liver toxicity may be caused by a reactive electrophile formed during metabolism (Lamb et al. 1986) since the liver has the enzymes necessary for activation. Results from single-dose oral studies have shown that biliary epithelial cells are earlier toxicity targets than liver parenchymal cells (Bailie et al. 1994), but the exact mechanism involved is unknown. A recent study showed that bile is a major route of biliary epithelial cell exposure to proximate toxicants of 4,4′-methylenedianiline (Kanz et al. 1995). This conclusion was based on the fact that rats infused through their common bile duct with bile from rats treated with 4,4′-methylenedianiline exhibited a much greater percentage of necrosis in the common bile duct than rats infused with control bile. The mechanism of thyroid toxicity has not yet been resolved. 4,4′-Methylenedianiline induced a slight decrease in serum T_3 and T, in rats (Hiasa et al. 1984). This decrease, according to the investigators (Hiasa et al.1984), may have triggered secretion of thyroid stimulating hormone (TSH), which in turn induced thyroid hyperplasia.

4,4′-Methylenedianiline was carcinogenic in rats and mice administered via drinking water (Lamb et al. 1986; NTP 1983). Although the mechanism of carcinogenicity is not known, some investigators

speculated that, at least for the liver, carcinogenicity may be related to the formation of a reactive metabolic intermediate which could bind to DNA (Lamb et al. 1986). However, a nongenetic mechanism of liver cancer is supported by recent data showing a relatively low potential of 4,4′-methylenedianiline to bind to rat liver DNA *in vivo* (Schiitze et al. 1996; Vock et al. 1996). As for thyroid cancer, some investigators believe that the goitrogenic activity of 4,4′-methylenedianiline supports a nongenetic mechanism (Lamb et al. 1986). Therefore, the appearance of liver and thyroid tumors may be the consequence of chronic tissue-damaging or tissue-stimulating effects, respectively, of 4,4′-methylenedianiline. 4,4′-Methylenedianiline inhibited neoplastic responses in the liver, kidney, and urinary bladder in initiated rats (Fukushima et al. 1981; Masui et al. 1986). The mechanism is not known but, according to the investigators, it may be related to a reduction in food consumption and, consequently, reduced growth. 4,4′-Methylenedianiline promoted the development of thyroid tumors in rats (Hiasa et al. 1984); the authors speculated that hypersecretion of TSH may have contributed to tumor formation in initiated cells.

2.4.3 Animal-to-Human Extrapolations

The limited information available shows that N-acetylation of 4,4′-methylenedianiline is a metabolic reaction shared by humans and animals. N-acetylmethylenedianiline has been detected in the urine of rats exposed by the oral route (Tanaka et al. 1985) and in humans exposed to 4,4′-methylenedianiline in the workplace (Cocker et al. 1986a, 1994). However, an attempt to discuss potential interspecies differences or similarities in 4,4′-methylenedianiline toxicity based solely on this information seems inappropriate at this time.

2.5 RELEVANCE TO PUBLIC HEALTH

Overview.

Most of the information on human health effects of 4,4′-methylenedianiline emanates from a case of ingestion of contaminated food and several reports of exposure in the workplace. The inhalation and dermal routes represent the most likely routes of exposure to 4,4′-methylenedianiline in occupational settings. Exposure to 4,4′-methylenedianiline for the general population seems unlikely. Case reports generally have many limitations, including lack of precise exposure data and presence of other compounds to which individuals may have been exposed, as well as confounding factors. The

presence of liver effects appears to be well established in workers exposed to 4,4′-methylenedianiline and in subjects who ingested food contaminated with 4,4′-methylenedianiline. Dermal effects also have been reported in occupationally exposed humans. There are also reports of respiratory, cardiovascular, hematological, and renal effects in exposed humans, but the evidence is not strong enough to conclusively establish cause-effect relationships. Results from one study were also inconclusive with respect to 4,4′-methylenedianiline and cancer in humans. 4,4′-Methylenedianiline was carcinogenic in rats and mice. Studies in animals are consistent and supportive of the human data with regard to liver effects. In addition to liver effects, the thyroid also appears to be a target for 4,4′-methylenedianiline toxicity in animals. No thyroid effects have been reported in humans, but this end point has not been appropriately evaluated. Little is known about toxicokinetics of 4,4′-methylenedianiline in humans. 4,4′-Methylenedianiline has been detected in the urine of workers exposed to the chemical. Animals can absorb 4,4′-methylenedianiline through inhalation, ingestion, or skin contact with the chemical. Methods are currently being developed to assess exposure by quantitatively determining 4,4′-methylenedianiline adducts to hemoglobin. Because 4,4′-methylenedianiline is a liver toxicant, individuals with compromised liver function may be at a greater risk. Based on limited data regarding environmental exposure, the most likely exposure route for populations living near hazardous waste sites is the dermal route. This route may be of concern to humans since animal studies have shown that 4,4′-methylenedianiline which remains within the skin after washing with soap and water is a potential source for later absorption.

Minimal Risk Levels for 4,4′-Methylenedianiline.

Inhalation MRLs.

No inhalation MRLs were derived for 4,4′-methylenedianiline due to lack of human and animal data.

Oral MRLs.

- An MRL of 0.2 mg/kg/day has been derived for acute oral exposure (14 days or less) to 4,4′-methylenedianiline.

The acute oral MRL is based on a minimal LOAEL of 25 mg/kg for liver toxicity in rats administered a single dose of 0 (controls), 25, 50, 75, 100, 125, or 225 mg 4,4′-methylenedianiline/kg by gavage in corn

oil (Bailie et al. 1993). The minimal effective dose was between 25 and 75 mg/kg. The severity of the effects was dose-related. Effects observed included increased serum alanine aminotransferase and gamma-glutamyl transferase, increased serum bilirubin, decreased bile flow, and increased relative liver weight. All these effects were indicative of hepatic parenchymal injury. A dose of 100 mg/kg caused hepatocellular necrosis, bile ductular necrosis, portal edema, and neutrophil infiltration. Bile ductular necrosis was observed 4 hours after dosing, and this was the earliest change identified. These finding are supported by those of Bailie et al. (1994) and Schmidt et al. (1980), who also observed liver damage in rats after a single dose of 50 mg 4,4′-methylenedianiline. It has also been shown that the liver is a target for 4,4′-methylenedianiline toxicity in humans by the oral and dermal routes, and in animals by the dermal route.

- An MRL of 0.08 mg/kg/day has been derived for intermediate oral exposure (15-364 days) to 4,4′-methylenedianiline.

The intermediate oral MRL is based on a NOAEL of 8.3 mg/kg/day for liver effects in rats administered daily doses of 4,4′-methylenedianiline by gavage in propylene glycol for 12 weeks (Pludro et al. 1969). Effects observed at a higher dose of 83 mg/kg/day included increased relative weight of the liver and kidney, atrophy of the liver parenchyma with hyperplasia of the stroma, particularly at portal areas, and increased serum beta-globulin and decreased albumin. Many studies have identified LOAELs in the range of 67-100 mg/kg/day, which is consistent with the results of Pludro et al. (1969). However, most of these studies tested only one dose level. A study conducted by NTP (1983) defined hepatic NOAELs of 35 mg/kg/day and 58 mg/kg/day for rats and mice, respectively.

No chronic oral MRL was derived for 4,4′-methylenedianiline. The lowest LOAEL for a number of end points was 2.7 mg/kg/day in a study in which dogs were treated for 54-84 months with 4,4′-methylenedianiline in a capsule 3 days per week (Deichmann et al. 1978). However, this study was not welldesigned and was poorly reported, which greatly diminished the potential significance of the results. No other study in dogs that could support the results of Deichmann et al. (1978) was located.

Death. No studies were located that reported deaths in humans attributable to exposure to 4,4′-methylenedianiline. In a population that had consumed bread made with flour contaminated with

4,4′-methylenedianiline in 1965 (Kopelman et al. 1966) the causes of death over the next 20 years were unremarkable (Hall et al. 1992). Observed/expected ratios for specific illnesses were well below 1.0. Data from acute, intermediate, and chronic-duration oral exposure in animals suggest that mice are more sensitive than rats (Griswold et al. 1968; Lamb et al. 1986; NTP 1983). Decreased survival rates were observed in mice treated with 36 mg 4,4′-methylenedianiline kg/day for 30 days (Griswold et al. 1968) and in mice treated with 57 mg/kg/day for 103 weeks (Lamb et al. 1986; NTP 1983). Mice were also more sensitive to acute dermal doses of 4,4′-methylenedianiline than rabbits (DuPont 1976a; Holland et al. 1987). Although environmental data are limited, it is unlikely that 4,4′-methylenedianiline levels near hazardous waste sites are sufficient to cause death in exposed populations after single or few exposures. The limited data in animals are inconclusive in determining whether prolonged low-level exposures in humans may represent a health hazard.

Systemic Effects.

Respiratory Effects. No respiratory effects were observed in a population that became ill after ingesting bread contaminated with 4,4′-methylenedianiline (Kopelman et al. 1966), and no studies were located that described respiratory effects in subjects exposed to 4,4′-methylenedianiline in the workplace. The respiratory system was not a target for 4,4′-methylenedianiline toxicity in animals by the inhalation (Leong et al. 1987), oral (NTP 1983) or dermal (DuPont 1975) route. For populations near Superfund sites, the potential for developing adverse respiratory effects from exposure to 4,4′-methylenedianiline seems unlikely.

Cardiovascular Effects. Lingering myocardial abnormalities were reported in a subject that had dermal contact with 4,4′-methylenedianiline in the workplace during a period of 2 weeks (Brooks et al. 1979). However, no information was provided regarding possible simultaneous exposure to other chemicals or past medical history. Furthermore, no acute or chronic signs of cardiovascular disease were observed in a population that was exposed to 4,4′-methylenedianiline by eating contaminated bread (Kopelman et al. 1966; Roy et al. 1985). The cardiovascular system was not a target for 4,4′-methylenedianiline toxicity in animals exposed orally in intermediate- or chronic-duration studies (Lamb et al. 1986; NTP 1983). There is insufficient evidence to assess the risk of cardiovascular disease in populations exposed to low levels of 4,4′-methylenedianiline for a prolonged period of time.

Gastrointestinal Effects. No acute or chronic signs of gastrointestinal disease were observed in a population that ate bread contaminated with 4,4′-methylenedianiline (Kopelman et al. 1966; Roy et al. 1985); however, nausea, abdominal pain, and vomiting were experienced by a subject who ingested 4,4′-methylenedianiline in an alcoholic beverage (Tillmann et al. 1997). Gross abnormalities were observed in the stomach of female rats treated with ≥261 mg 4,4′-methylenedianiline in the drinking water for 14 days (NTP 1983). However, the significance of this finding is unclear since this effect was not seen in males treated in a similar manner with doses ≥235 mg/kg/day, although lesions were evident at 469 mg/kg/day (NTP 1983). Oral intermediate or chronic-duration studies in animals showed no adverse effects in the gastrointestinal tract after administration of 4,4′-methylenedianiline. The evidence in animals suggest that the gastrointestinal tract is not a target for 4,4′-methylenedianiline toxicity, but the human data are inconclusive as to the potential effects of prolonged oral exposure to 4,4′-methylenedianiline.

Hematological Effects. Eosinophilia was described in a subject who accidentally ingested a solution containing 4,4′-methylenedianiline (Roy et al. 1985). Other components in the solution were potassium carbonate and gamma-butyrolactone. Mild leucocyte elevation was reported in a young man who ingested an undetermined amount of 4,4′-methylenedianiline mixed in an alcoholic beverage (Tillmann et al. 1997). Eosinophilia was also observed in four of six men who became in contact with 4,4′-methylenedianiline at work (Williams et al. 1974). Eosinophilia is a characteristic response observed in allergic reactions; therefore, in these cases it was most likely caused by exposure to 4,4′-methylenedianiline, even though other chemicals may have been involved. No significant hematological effects have been reported in animals after exposure to 4,4′-methylenedianiline; however, this system has not been appropriately evaluated. The exact role of eosinophils in the allergic immunological reaction is not known. The existing information suggests that eosinophilia may develop after exposure to 4,4′-methylenedianiline as may occur in populations living near hazardous waste sites.

Musculoskeletal Effects. The only information regarding musculoskeletal effects in humans after exposure to 4,4′-methylenedianiline is from a case report of a young man who complained of joint and muscle pain on admission to the hospital one day after drinking an alcoholic beverage spiked with an unknown amount of 4,4′-methylenedianiline (Tillmann et al. 1997). Long-term oral studies in both rats and mice found no evidence of gross or histopathological alterations in the musculoskeletal system

(Lamb et al. 1986; NTP 1983). Based on this limited evidence, no predictions can be made regarding musculoskeletal effecIs in populations exposed to 4,4′-methylenedianiline.

Hepatic Effects. It is well established that 4,4′-methylenedianiline is a liver toxicant in humans (Bastian 1984; Brooks et al. 1979; Kopelman et al. 1966; McGill and Motto 1974; Tillmann et al. 1997; Williams et al. 1974) and animals (Bailie et al. 1993, 1994; Fukushima et al. 1981; Kanz et al. 1992; Lamb et al. 1986; NTP 1983) regardless of the route of exposure. In humans that developed toxic hepatitis as a result of accidentally ingesting 4,4′-methylenedianiline (Kopelman et al. 1966), there was no sign of progressive hepatic disease upon examination two years after the poisoning episode (Kopelman 1968). No deaths attributed to liver disease were reported among this same population over a period of 20 years after the accident (Ball et al. 1992). In animals, the severity of the effects was generally dose-related, and results from single dose studies showed that biliary epithelial cells are affected earlier than parenchymal liver cells (Bailie et al. 1994) and that the toxicant responsible for the damage is present in the bile (Kanz et al. 1995), although its chemical nature has not been established. It was also shown that leukocyte infiltration observed in the liver after an oral dose of 4,4′-methylenedianiline does not contribute to liver damage. Acute-duration studies, mainly in rats, identified minimal effective doses around 25 mg/kg/day (Bailie et al. 1993). A NOAEL of 8.3 mg/kg/day was established in intermediate-duration study in rats (Pludro et al. 1969); LOAELs ranged between 67 and 100 mg/kg/day. Chronic LOAELs were 9 and 25 mg/kg/day in rats and mice, respectively (Lamb et al. 1986; NTP 1983). A LOAEL of 2.7 mg/kg/day was reported in dogs, but this study had severe design limitations (Deichmann et al. 1978). An acute oral MRL of 0.2 mg/kg/day was derived based on the results of a study by Bailie et al. (1993). In deriving this MRL, a modifying actor of 0.5 was used to account for the possibility that the corn oil vehicle might have facilitated the absorption of 4,4′-methylenedianiline in the gastrointestinal tract. An intermediate MRL of 0.08 mg/kg/day was based on results from Pludro et al. (1969). The exact mechanism involved in liver toxicity is unknown, but some have suggested that it may be related to the formation of a reactive electrophile as a result of metabolic activation of 4,4′-methylenedianiline (Lamb et al. 1986). The existing evidence indicates that humans exposed to elevated levels of 4,4′-methylenedianiline, which may be found in the workplace, may be at risk of developing liver disease.

Endocrine Effects. No information was located regarding endocrine effects in humans exposed to 4,4′-methylenedianiline by any route. Results from oral studies in animals suggest that the thyroid is a target for 4,4′-methylenedianiline toxicity. Gross and histopathological alterations have been described

in rats and mice in acute- (Tullner 1960), intermediate- (Fukushima et al. 1981; Hagiwara et al. 1993; Hiasa et al. 1984; NTP 1983; Tsuda et al. 1987), and chronic-duration (Lamb et al. 1986; NTP 1983) oral studies. The mechanism of thyroid toxicity is not known, but some have speculated that it may be related to induced hypersecretion of thyroid stimulating hormone (TSH) which in turn induces thyroid hyperplasia (Hiasa et al. 1984). The relevance of the thyroid effects observed in animals to human health is unknown. Thyroid function has not been examined in populations with known exposure to 4,4′-methylenedianiline.

Renal Effects. Results from clinical tests in a subject who accidentally drank a solution containing 4,4′-methylenedianiline, potassium carbonate, and gamma-butyrolactone were consistent with renal tubular dysfunction (Roy et al. 1985), but the role of 4,4′-methylenedianiline, if any, is unknown. The only other relevant information in humans is a case in which proteinuria and erythrocyturia were observed in a young man who drank an alcoholic beverage spiked with 4,4′-methylenedianiline (Tillmann et al. 1997). No further data in humans were located. Intermediate-duration oral studies in animals established NOAELs of 83-141 mg/kg/day (Fukushima et al. 1979, 1981; NTP 1983; Pludro et al. 1969). However, in chronic-duration oral studies, high incidence of nephropathy was reported in mice at 19-25 mg 4,4′-methylenedianiline/kg/day and a high incidence of kidney mineralization was seen in male rats at 16 mg/kg/day (Lamb et al. 1986; NTP 1983). A much lower LOAEL of 2.7 mg/kg/day was reported in dogs, but this study suffered from severe limitations (Deichmann et al. 1978). The results from these long-term studies in animals suggest that the possibility exists for similar effects to occur in humans exposed to low 4,4′-methylenedianiline levels for a prolonged period of time, such as can occur near Superfund waste sites.

Dermal Effects. Erythema multiform was observed in a subject who accidentally ingested a liquid containing 4,4′-methylenedianiline, potassium carbonate, and gamma-butyrolactone (Roy et al. 1985). Although more than one chemical was involved in this case, the presence of erythema multiform is consistent with an immunological allergic reaction. A recent study reported that a subject who drank an alcoholic beverage spiked with 4,4′-methylenedianiline developed a skin rash (Tillmann et al. 1997). Skin rashes have commonly been described in individuals who had dermal contact with 4,4′-methylenedianiline (Brooks et al. 1979; Emmett 1976; McGill and Motto 1974; Van Joost et al. 1987). Also, a case of photosensitivity to 4,4′-methylenedianiline was described (Levine 1983). Exposure concentrations were not available. In some cases (Brooks et al. 1979; McGill and Motto 1974), skin rashes and pruritus could have been a manifestation of the underlying liver disease.

However, in other cases (Emmett 1976; Van Joost et al. 1987), proof of dermal sensitization was obtained when patch testing with 4,4′-methylenedianiline gave positive results. In the case described by Van Joost et al. (1987), the patient had no known previous exposure to 4,4′-methylenedianiline, but had been exposed to other chemicals. This led the investigators to suggest that cross sensitization may have occurred, specifically, to agents with substitution in the para position. The possibility of dermal sensitization across routes of exposure was examined in guinea pigs and the results were negative (Leong et al. 1987). The existing evidence indicates that 4,4′-methylenedianiline can cause dermal sensitization in humans.

Ocular Effects. Prolonged and severe loss of visual acuity was reported in a subject who accidentally ingested a liquid containing 4,4′-methylenedianiline, potassium carbonate, and gamma-butyrolactone (Roy et al. 1985). These effects could not, however, be conclusively attributed to 4,4′-methylenedianiline. No other reports were found regarding ocular effects in humans after exposure to 4,4′-methylenedianiline. Supporting the findings of Roy et al. (1985) is a report in which guinea pigs exposed nose-only to an aerosol of 4,4′-methylenedianiline for 2 weeks had serious morphological alterations in the retina (Leong et al. 1987). It is assumed that nose-only exposure prevented direct contact of the aerosol with the eye. On the other hand, no specific complaints of ocular effects were observed among a group of individuals who ate bread contaminated with 4,4′-methylenedianiline and developed toxic hepatitis (Kopelman 1968; Kopelman et al. 1966). Deposition of solid 4,4′-methylenedianiline into the eye of rabbits induced acute reversible effects in the cornea, iris, and conjunctiva (DuPont 1976b). The evidence available suggests that populations living near Superfund waste sites are not at a greater risk of developing adverse ocular effects due to exposure to 4,4′-methylenedianiline. However, direct contact with concentrated material, as may occur occupationally, can be hazardous.

Body Weight Effects. *No* studies were located regarding body weight effects in humans exposed to 4,4′-methylenedianiline by any route. Acute (NTP 1983), intermediate (Fukushima et al. 1979, 1981; Hagiwara et al. 1993; Hiasa et al. 1984; Miyamoto et al. 1977; NTP 1983; Tsuda et al. 1987), or chronic (Lamb et al. 1986; NTP 1983) oral exposure to 4,4′-methylenedianiline in animals usually resulted in a reduction in final body weight. Similar results were reported in rabbits exposed dermally for 10 days (DuPont 1976a). Food consumption data were not provided in these studies. The relevance of these findings to human health is unknown.

Immunological and Lymphoreticular Effects. Several cases of dermal sensitization have been described in subjects who became in contact with 4,4′-methylenedianiline in the workplace (Emmett 1976; Levine 1983; Van Joost et al. 1987), but no information exists regarding possible effects of 4,4′-methylenedianiline on human immunocompetence. Studies in animals have described histopathological alterations in the thymus of rats after single oral doses of 250 mg 4,4′-methylenedianiline/kg (Kanz et al. 1992). No histopathological alterations were seen in organs of the lymphoreticular system from rats or mice in intermediate or chronic oral studies (Lamb et al. 1986; NTP 1983). Immunocompetence has not been evaluated in animals. There is insufficient information to assess the potential immunological effects of prolonged exposure to low levels of 4,4′-methylenedianiline in humans.

Neurological Effects. No studies were located regarding neurological effects in humans after exposure to 4,4′-methylenedianiline by any route. Studies in animals provided no evidence of neurotoxicity after intermediate or chronic exposure (Lamb et al. 1986; NTP 1983), although a complete neurological examination including neurobehavioral testing has not been performed. Because 4,4′-methylenedianiline is a solid with low volatility and because of what is known about structurally similar compounds, it is not anticipated that exposures around Superfund sites will cause neurological damage in humans.

Reproductive Effects. No studies were located regarding reproductive effects in humans after exposure to 4,4′-methylenedianiline by any route. Hypertrophy of the uterus was observed in rats treated orally for 5-14 days with 110 mg 4,4′-methylenedianiline/kg/day (Tullner 1960). However, no such effects were seen after exposure to similar daily doses for 13 weeks (NTP 1983) or to lower doses for up to 103 weeks (Lamb et al. 1986; NTP 1983). Endometrial hyperplasia was reported in a dogs treated with doses of about 2.7 mg 4,4′-methylenedianiline/kg/day for over 54 months, but this study had severe design limitations (Deichmann et al. 1978). None of the animal studies examined reproductive function. There is not enough information to predict whether reproductive function in humans might be affected after exposure to 4,4′-methylenedianiline.

Developmental Effects. No studies were located regarding developmental effects in humans after exposure to 4,4′-methylenedianiline by any route. A single oral study in rats administered 4,4′-methylenedianiline during gestation showed that doses ≥37 mg/kg/day may cause developmental abnormalities in the offspring (Bourdelat et al. 1983). It should be noted, however, that these dose

levels also induced maternal toxicity. Furthermore, only one rat was used as control and the results were poorly reported. Based on the evidence available, no predictions can be made regarding developmental effects in populations exposed to 4,4′-methylenedianiline.

Genotoxic Effects. As seen in Table 2-4, limited information exists regarding genotoxic effects of 4,4′-methylenedianiline *in vivo*. In rats, a single intraperitoneal injection of approximately 73 mg 4,4′-methylenedianiline/ significantly increased the incidence of DNA fragmentation in liver cells (Parodi et al. 1981). In a study in which only two rats were used, intraperitoneal administration of approximately 80 mg 4,4′-methylenedianiline/kg to one rat increased the amount of DNA adducts in the liver relative to the control rat treated with the vehicle only (Endo and Hara 1991). In more recent studies, it was shown that 4,4′-methylenedianiline has a relatively low DNA binding potency (Schiitze et al. 1996). In that study, the total radioactivity bound to liver DNA from rats treated with a single intraperitoneal dose of 0.2 or 23 mg of 4,4′-methylenedianiline/kg corresponded to 0.06 and 2.7 adducts per 10.' nucleotides, and this put 4,4′-methylenedianiline in the range of weakly genotoxic compounds. The structure of the adducts was not determined, but it was determined that adduct formation was covalent in nature. Similar results were reported in rats after acute oral administration of 4,4′-methylenedianiline (Vock et al. 1996). In the latter case, no adducts could be detected in the bladder and peripheral lymphocytes. Contradictory results were obtained regarding sex-linked recessive lethal mutations in *Drosophila melanogaster*. Foureman et al. (1994) reported a significant increase in the incidence of recessive lethal mutations while Ho et al. (1979) reported no significant increase.

Many studies have examined the genotoxic properties of 4,4′-methylenedianiline *in vitro* in *Salmonella typhimurium* (Andersen et al. 1980; Cocker et al. 1986a; Ho et al. 1979; LaVoie et al. 1979; McCarthy et al. 1982; Messerly et al. 1987; Parodi et al. 1981; Rannug et al. 1984; Rao et al. 1982; Takemura and Shimizu 1978; Tanaka et al. 1985; Tsuchiya 1995). With few exceptions (see Table 2-5), the results showed that 4,4′-methylenedianiline is genotoxic after metabolic activation with S-9 systems. Although not conclusively established, the genotoxic properties have been attributed to a reactive electrophile formed as a result of N-hydroxylation. On the other hand, N-acetylated metabolites have been shown to be nonmutagenic (Cocker et al. 1986b; Tanaka et al. 1985). 4,4′-Methylenedianiline also was shown to be mutagenic in the eukaryotic organism *Saccharomyces cerevisiae* with and without metabolic activation (Ho et al. 1979). 4,4′-Methylenedianiline did not increase the incidence of sister chromatid exchanges in human leukocytes *in vitro* when tested with or without activation (Ho

Table 2-4. Genotoxicity of 4,4′-Methylenedianiline *In Vivo*

Species (test system)	End point	Results	Reference
Rat liver cells	DNA fragmentation	+	Parodi et al. 1981
Mouse bone marrow cells	Sister chromatid exchange	+	Parodi et al. 1981
Drosophila melanogaster	Sex linked recessive lethal mutations	+	Foureman et al. 1994
D. melanogaster	Sex linked recessive lethal mutations	–	Ho et al. 1979

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

Table 2-5. Genotoxicity of 4,4′-Methylenedianiline *In Vitro*

Species (test system)	End point	Result: With activation	Result: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium TA 100 on plates	Reverse mutation	+	ND	Andersen et al. 1980
		+	ND	Parodi et al. 1981
		+	ND	McCarthy et al. 1982
		+	ND	Cocker et al. 1986b
		+	–	Takemora and Shimizu 1978
		+	–	LaVoi et al. 1979
		+	–	Tanaka et al. 1985
		+	–	Rao et al. 1982
		+	–	Messerly et al. 1987
		+	–	Tsuchiya 1995
S. typhimurium TA 98 on plates	Reverse mutation	–	ND	Parodi et al. 1981
		–	–	Takemora and Shimizu 1978
		+	–	Tanaka et al. 1985
		+	–	Rao et al. 1982
		+	–	Rannug et al. 1984
		(+)	–	Messerly et al. 1987
		–	–	Tsuchiya 1995
S. typhimurium TA 1538, TA 1535 on plates	Reverse mutation	+	–	Rannug et al. 1984
Eukaryotic organisms: *Saccharomyces cerevisiae*	DNA repair	+	+	Ho et al. 1979
Mammalian cells		ND	+	Mori et al. 1988
Rat hepatocytes	Unscheduled DNA synthesis			
Rat hepatocytes	Unscheduled DNA synthesis	–	+	Mirsalis et al. 1983
L5178Y mouse lymphoma cells	Forward mutation	ND	+	McGregor et al. 1988
Human leukocytes	Sister chromatid exchange	–	–	Ho et al. 1979

– = negative results; + = positive results; (+) = weakly positive results; DNA = deoxyribonucleic acid; ND = no data

et al. 1979), but increased unscheduled DNA synthesis in rat hepatocytes (Mirsalis et al. 1983; Mori et al. 1988) and the incidence of forward mutations in L5178Y mouse lymphoma cells (McGregor et al. 1988). The overall evidence is insufficient to indicate that exposure to 4,4′-methylenedianiline may cause genetic damage in humans.

Cancer. Limited information exists regarding potential carcinogenicity of 4,4′-methylenedianiline in humans. No association was found between deaths from cancers and 4,4′-methylenedianiline in a group of 84 subjects who ate bread contaminated with 4,4′-methylenedianiline (Hall et al. 1992). The evaluation was conducted over a period of 20 years following the accident. However, the cohort is quite small for drawing any firm conclusions. Similar lack of association was reported for a cohort of 550 male and 45 female Swedish workers exposed to 4,4′-methylenedianiline (predominantly by dermal contact) (Selden et al. 1992). The exposure period was not clearly established. It was noted, however, that the subjects were quite young and had not reached cancer-prone age, and that the follow-up period was short and may not have covered the latency period for some cancers, in particular bladder cancer, which has a latency of about 20 years. Two additional studies also provided inconclusive evidence of increased incidence of bladder cancer among workers exposed to 4,4′-methylenedianiline; studies were inconclusive mainly because of simultaneous exposure to other chemicals (Cragle et al. 1992; Liss and Guirguis 1994).

Results from a 2-year drinking water bioassay provided clear evidence of carcinogenicity in rats and mice (Lamb et al. 1986; NTP 1983). Under the conditions of the study, 4,4′-methylenedianiline caused significantly increased incidences of thyroid follicular cell carcinomas in male rats, thyroid follicular cell adenomas in female rats and both male and female mice, C-cell adenomas of the thyroid gland in female rats, neoplastic nodules in the liver of male rats, hepatocellular carcinomas in male and female mice, adenomas of the liver and malignant lymphomas in female mice, and adrenal pheochromocytomas in male mice. It should be mentioned, however, that considerable debate exists on whether adenomas result in, or are a precursor to, malignant neoplasms.

4,4′-Methylenedianiline was not a promoter of liver, kidney, or urinary bladder tumors in rats (Fukushima et al. 1981; Masui et al. 1986); in fact, it inhibited the neoplastic response induced by the carcinogens alone. It was postulated that the mechanism may be related to a decreased food consumption and reduced growth (Fukushima et al. 1981; Masui et al. 1986). In contrast,

4,4′-methylenedianiline promoted the formation of thyroid tumors in rats initiated with N-bis(2-hydroxypropyl)nitrosamine (Hiasa et al. 1984).

The mechanism of 4,4′-methylenedianiline carcinogenicity is not known. It has been suggested that a reactive intermediate resulting from metabolic activation may be responsible for the liver carcinogenicity by binding to cell macromolecules, and that a nongenetic mechanism may be involved in thyroid carcinogenicity (Lamb et al. 1986). Based on recent data regarding the low potential of 4,4′-methylenedianiline to bind to rat liver DNA (Schiitze et al. 1996), a nongenotoxic mechanism of liver carcinogenicity would also appear likely. Based on inadequate evidence of carcinogenicity in humans and sufficient evidence in experimental animals, the International Agency for Research on Cancer (IARC 1987) has designated 4,4′-methylenedianiline a Group 2B carcinogen and possibly carcinogen in humans.

2.6 BIOMARKERS OF EXPOSURE AND EFFECT

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

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

essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 4,4′-methylenedianiline are discussed in Section 2.6.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by 4,4′-methylenedianiline are discussed in Section 2.6.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.8, Populations That Are Unusually Susceptible.

2.6.1 Biomarkers Used to Identify or Quantify Exposure to 4,4′-Methylenedianiline

Exposure to 4,4′-methylenedianiline in manufacturing and use has been assessed by monitoring 4,4′-methylenedianiline in the urine (Cocker et al. 1986a, 1994; Dalene et al. 1995; Schtitze et al. 1995). A metabolite of 4,4′-methylenedianiline, N-acetylmethylenedianiline, also has been quantified in urine (Cocker et al. 1986a; Schtitze et al. 1995). Quantification of these substances is performed by gas chromatography-mass spectrophotometry. Cocker et al. (1994) was able to show that when exposure to 4,4′-methylenedianiline occurred through inhalation, postshift urine samples contained higher concentrations of 4,4′-methylenedianiline than samples taken preshift next day. When exposure was thought to be through the dermal route, urine collected preshift next day had higher-concentration of 4,4′-methylenedianiline than samples taken immediately postshift on the day of exposure. These results suggested that pulmonary absorption is relatively fast such that peak excretion is reached at the end of the shift. In contrast, absorption through the skin was slower and maximum excretion occurred the next morning. N,N'-diacetylmethylenedianiline has also been found in the urine of exposed workers, but at a much lower concentration than the monoacetylated metabolite (Robert et al. 1995).

Little is known about the pharmacokinetics of 4,4′-methylenedianiline in humans, but data in animals suggest that it is eliminated fairly rapidly and does not accumulate. This is consistent with what has been found in occupationally exposed humans. In a study involving 411 exposed workers, 42% of the urine samples had no detectable 4,4′-methylenedianiline (Cocker et al. 1994). In another study of 111 exposed workers, the concentration of 4,4′-methylenedianiline in 81% of over 300 urine samples was below the detection limit for the method (Cocker et al. 1986a). This suggests that monitoring urinary 4,4′-methylenedianiline may not be sensitive enough in cases of brief or single exposures to low levels of the chemical. Furthermore, only an indication of recent exposure would be obtained. In contrast with the results from Cocker et al. (1986a), a recent study by Schtitze et al. (1995) found 4,4′-methylenedianiline in the urine from workers exposed to low levels of the chemical (<20 pg/m^3). Thirty-three workers were examined and 4,4′-methylenedianiline was detected in the urine (0.013-2.76 nmol/L) from all of them. Urinary levels of the metabolite N-acetylmethylenedianiline ranged from 0.045 to 23.4 nmol/L.

Recent studies have examined the formation of adducts of 4,4′-methylenedianiline with hemoglobin as a means for monitoring exposure (Bailey et al. 1990; Farmer and Bailey 1989; Schiitze et al. 1995). In the recent study by Schiitze et al. (1995), the authors showed that there was a good correlation (r=0.77) between adduct levels in hemoglobin and 4,4′-methylenedianiline released after acid treatment of urine. The following mechanism of adduct formation has been postulated (Farmer and Bailey 1989). N-hydroxymethylenedianiline resulting from the enzymatic oxidation of 4,4′-methylenedianiline is further oxidized to nitroso compounds, which may react with sulfhydryl groups in hemoglobin. This yields N-hydroxy sulphenamides, which then rearrange to more stable sulphinamides. Hydrolysis of the sulphinamides under mild acidic or basic conditions releases the parent amine, which may be extracted and quantitated. This approach has a clear advantage over directly monitoring 4,4′-methylenedianiline because the adduct is stable *in vivo* and its elimination is related to the lifespan of the erythrocytes, which in humans is 120 days (Farmer and Bailey 1989). In addition, there is an advantage over DNA adducts which are subjected to DNA repair reactions. Although no data exist yet in humans, studies in rats showed that 4,4′-methylenedianiline and N-acetylmethylenedia.niline formed adducts with hemoglobin and that a dose-response relationship could be established over the range of oral doses used (1-12 mg/kg) (Bailey et al. 1990).

2.6.2 Biomarkers Used to Characterize Effects Caused by 4,4′-Methylenedianiline

There are no specific biomarkers of effects for 4,4′-methylenedianiline. Studies summarized in Section 2.2 indicate that exposure to 4,4′-methylenedianiline causes adverse liver effects in humans (Kopelman et al. 1966; McGill and Motto 1974; Williams et al. 1974). However, exposure to many other chemicals, not even necessarily of a structure similar to 4,4′-methylenedianiline, can produce a similar clinical picture. Also, the properties of dermal sensitizer are not unique to 4,4′-methylenedianiline, and a positive patch testing response to 4,4′-methylenedianiline may also be obtained in cases of previous contact with chemicals substituted in the para-position (cross sensitization) (Van Joost et al. 1987).

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

2.7 INTERACTIONS WITH OTHER CHEMICALS

No information was located regarding the influence of other chemicals on the toxicity of 4,4′-methylenedianiline in humans. Limited data in animals showed that pretreatment of rats with the monooxygenase function inhibitor aminobenzotriazol, ameliorated the hepatic effects of 4,4′-methylenedianiline (Bailie et al. 1993). However, pretreatment with the inhibitor SKF-525A had no effect. Based on these results, the investigators proposed that 4,4′-methylenedianiline requires metabolic activation for its toxicity and that activation is carried out by an isozyme of cytochrome P-450 that is inhibited by aminobenzotriazol, but not SKF-525A (Bailie et al. 1993).

4,4′-Methylenedianiline inhibited the formation of liver, kidney, and urinary bladder tumors when administered orally to rats following initiation with various carcinogens (Fukushima et al. 1981; Masui et al. 1986). The mechanism for this interaction is not known. Some have suggested that a reduction in food consumption and, consequently, in growth, induced by 4,4′-methylenedianiline, may play a role (Fukushima et al. 1981). A different possibility that has been offered is that 4,4′-methylenedianiline may alter the activities of enzymes involved in carcinogen metabolism, resulting in reduced biotransformation of the carcinogens (Tsuda et al. 1987). 4,4′-Methylenedianiline has been shown to alter the activities of several biotransformation enzymes in rats (Wu et al. 1989).

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to 4,4′-methylenedianiline than will most persons exposed to the same level of 4,4′-methylenedianiline in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters may result in reduced detoxification or excretion of 4,4′-methylenedianiline, or compromised function of target organs affected by 4,4′-methylenedianiline. Populations who are at greater risk due to their unusually high exposure to 4,4′-methylenedianiline are discussed in Section 5.6, Populations With Potentially High Exposure.

In the review of the literature regarding toxic effects of 4,4′-methylenedianiline, no information was located on any population that might be unusually sensitive to 4,4′-methylenedianiline. However, based on the knowledge of the primary toxic effect of 4,4′-methylenedianiline (liver toxicity), a number of groups may be proposed as being potentially highly sensitive to this chemical. Some of these groups include very young children who have an immature hepatic detoxification system and individuals with impaired liver function (i.e., liver cirrhosis). In addition, people who develop dermatitis following exposure to 4,4′-methylenedianiline in the workplace may become hypersensitive to subsequent exposure to the chemical or to structurally related chemicals (Van Joost et al. 1987). Also, since the enzyme N-acetyltransferase exhibits polymorphism, slow acetylators will be prone to more toxic insult of 4,4′-methylenedianiline than fast acetylators, assuming that acetylation represents a true detoxification pathway.

2.9 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 4,4′-methylenedianiline. 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 4,4′-methylenedianiline. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

No texts were found that provided specific information about treatment following exposures to 4,4′-methylenedianiline.

2.9.1 Reducing Peak Absorption Following Exposure

4,4′-Methylenedianiline can be absorbed through the skin, by inhalation of fine dust or vapor, or by ingestion. The only recommendation found in the literature to reduce peak absorption after ingestion was induction of vomiting (Allied Chemical 1978). Removing the patient to fresh air is suggested if inhalation occurs, and washing with soap and water is recommended after contact with the skin (Allied Chemical 1978; NIOSH 1997). NIOSH (1997) further recommends immediately washing the eyes with large amounts of water, occasionally lifting the lower and upper lids. A recent study examined the efficacy of either 100% ethanol, 100% water, or 1% or 10% soap solution in removing 4,4′-methylenedianiline from human and rat skin *in vitro* (Hewitt et al. 1995). The results showed that all solutions were equally effective in removing the chemical from the human skin surface, with 2 l-47% of the applied dose removed at 72 hours. In contrast, 100% ethanol or 10% soap solution were significantly more effective in removin, 0 4,4′-methylenedianiline from the rat skin than 100% water or 1% soap solution. The results also showed that washing the skin surface 3 or 30 minutes after dosing significantly reduced absorption (2- to 3-fold) compared with control unwashed skin. Washing after 72 hours following application of the compound did not significantly reduce absorption. Data from an *in vivo* study in a groups of rats and guinea pigs and in a monkey showed that washing the application site with soap and water was more effective in removing 4,4′-methylenedianiline from the skin than washing with acetone and water (El-Hawaii et al. 1986).

2.9.2 Reducing Body Burden

No information was located regarding reducing the body burden after exposure to 4,4′-methylenedianiline.

2.9.3 Interfering with the Mechanism of Action for Toxic Effects

The mechanism of 4,4′-methylenedianiline toxicity is not lmown. Information on structurally related chemicals suggests that the toxicity of 4,4′-methylenedianiline is due to the formation of a reactive intermediate that can bind covalently to cell macromolecule. The reactive intermediate is produced by the enzymatic N-oxidation of 4,4′-methylenedianiline (Lamb et al. 1986). A different metabolic pathway, which has been demonstrated in humans, is N-acetylation (Cocker et al. 1986a, 1994). This pathway seems to represent a detoxification route since N-acetylmethylenedianiline and

N,N'-diacetylmethylenedianiline, in the presence of activating systems, were not genotoxic in mutagenicity assays (Cocker et al. 1986b; Tanaka et al. 1985). This was in contrast with a strong mutagenic effect of the parent compound with activation. Therefore, it would appear that if the acetylation pathway could be favored over the N-oxidation route, some toxic effects attributed to 4,4′-methylenedianiline might be diminished.

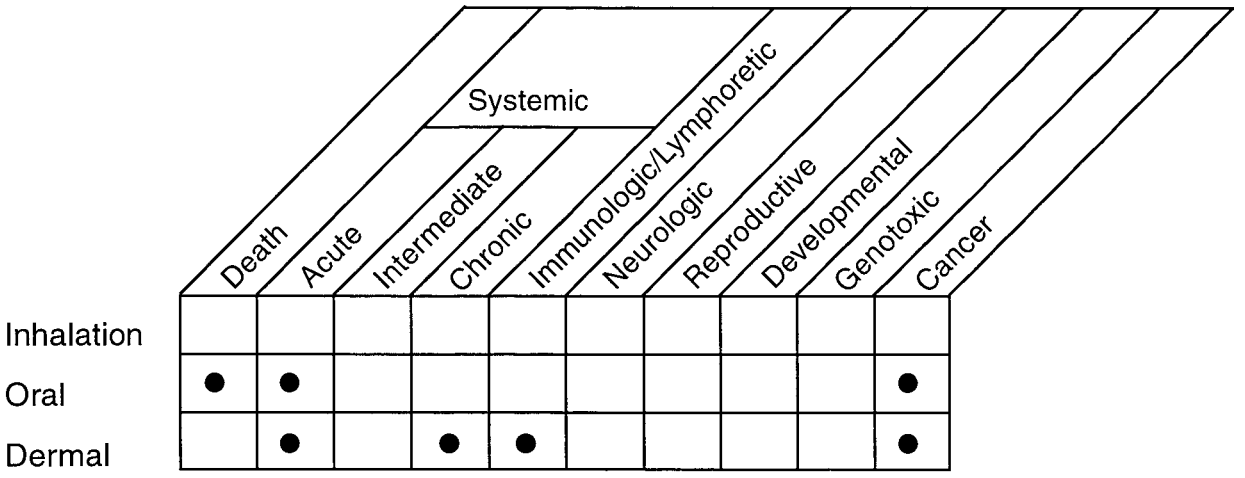
2.10 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 4,4′-methylenedianiline 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 4,4′-methylenedianiline.
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

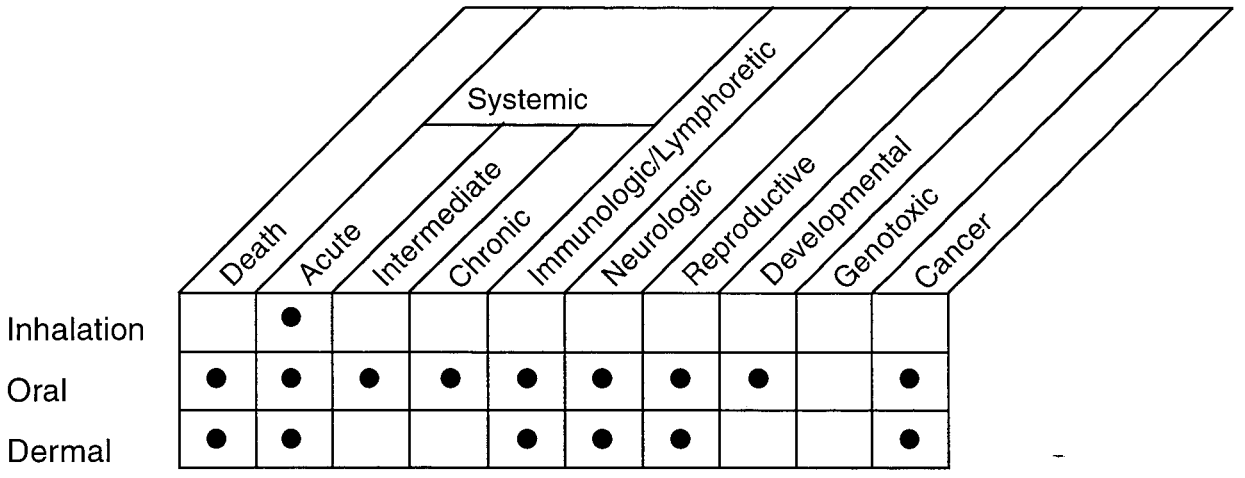
2.10.1 Existing information on Health Effects of 4,4′-Methylenedianiline

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 4,4′-methylenedianiline are summarized in Figure 2-5. The purpose of this figure is to illustrate the existing information concerning the health effects of 4,4′-methylenedianiline. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should-missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 2-5. Existing Information on Health Effects of 4,4'-Methylenedianiline



Human



Animal

● Existing Studies

Data regarding health effects of 4,4′-methylenedianiline in humans result from a few studies involving accidental acute oral exposure to 4,4′-methylenedianiline, and from cases of dermal exposure in the workplace. No information was located regarding inhalation exposure in humans. No information is available regarding neurological, reproductive, or developmental effects in humans by any route of exposure.

Most of the data regarding the health effects of 4,4′-methylenedianiline in animals were obtained from studies in which 4,4′-methylenedianiline was administered orally. A single study was located concerning health effects in animals following inhalation exposure, and few reports on dermal exposure to 4,4′-methylenedianiline were located. The available information from animal studies is insufficient to determine with certainty whether the effects of 4,4′-methylenedianiline are route- or species-specific, or age-dependent. Information on the dermal route of exposure would be useful because of the potential exposure via this route for workers in industry. Because of its low volatility, inhalation of 4,4′-methylenedianiline appears less likely than dermal contact, but cannot be completely ruled out. Although there are limited environmental data regarding 4,4′-methylenedianiline, the dermal route of exposure appears to be the most relevant for humans living near hazardous waste sites where 4,4′-methylenedianiline might be found.

2.10.2 Identification of Data Needs

Acute-Duration Exposure Populations living in the vicinity of hazardous waste sites may be exposed to 4,4′-methylenedianiline for a short time. Exposure would probably occur via the dermal route, but oral exposure, particularly by children ingesting contaminated soil, cannot be excluded.

The liver is the major target of 4,4′-methylenedianiline toxicity in humans following acute exposure by any route (Brooks et al. 1979; Kopelman et al. 1966; McGill and Motto 1974; Roy et al. 1985; Tillmann et al. 1997). The same was seen in rats (Bailie et al. 1993, 1994; Kanz et al. 1992; Schmidt et al. 1980), but other animal species have not been studied. An acute oral MRL was derived based on liver effects in rats identified in the Bailie et al. (1993) study. The mechanism of liver toxicity has not been elucidated, but it seems that the biliary tree epithelium is affected before the liver parenchyma (Bailie et al. 1994). Further studies are necessary to corroborate this finding and possibly identify the chemical entity responsible for the liver toxicity. In addition, studies providing quantitative estimates of excretion of 4,4′-methylenedianiline and/or metabolites in the bile would help

establish a possible relationship between this excretory route and bile duct toxicity. The existing information also showed that 4,4′-methylenedianiline can cause adverse dermal reactions in humans (Emmett 1976; Van Joost et al. 1987; Williams et al. 1974). Intermediate- and chronic-duration studies in animals suggest that the thyroid is also a target for 4,4′-methylenedianiline toxicity, but this end point has not been examined in acute-duration studies. Reproductive function has not been examined in acute-duration studies by any route; such studies could provide valuable information on possible effects of 4,4′-methylenedianiline on that system. Based on its chemical structure, 4,4′-methylenedianiline does not appear to be a neurotoxicant, but the nervous system has not been examined in acute-duration studies. A limited number of acute dermal studies in animals have suggested that the administration vehicle plays a role in the toxicity of 4,4′-methylenedianiline, possibly by influencing absorption (DuPont 1975, 1976a). Further dermal studies with different vehicles and also oral studies with 4,4′-methylenedianiline in different soils would provide valuable information for those living near hazardous waste sites where 4,4′-methylenedianiline may be found.

Intermediate-Duration Exposure. No studies were located on intermediate-duration exposure to 4,4′-methylenedianiline in humans by any route. The preponderance of data is available from rats exposed to 4,4′-methylenedianiline in the diet (Fukushima et al. 1979, 1981; Hagiwara et al. 1993; Hiasa et al. 1984; Miyamoto et al. 1977), by gavage (Pludro et al. 1969), or through the drinking water (NTP 1983). Results from these studies indicate that the liver and the thyroid are targets for 4,4′-methylenedianiline toxicity. An intermediate oral MRL was derived based on liver effects in rats reported by Pludro et al. (1969). Studies in other species would provide information on whether these end points are sensitive targets across species. The mechanism of thyroid toxicity is not known; therefore, research aimed to investigate this subject would be helpful. Neurological, reproductive, and developmental effects have not been appropriately studied in oral intermediate-duration studies, and no information is available regarding the inhalation and dermal routes of exposure. Pharmacokinetic data to help identify potential target organs after exposure by any route were not located.

Chronic-Duration Exposure and Cancer. No studies were located following chronic-duration inhalation or oral exposure to 4,4′-methylenedianiline in humans. Data were available concerning dermal exposure to 4,4′-methylenedianiline in the workplace. Results from one study confirmed that the liver is a target for 4,4′-methylenedianiline toxicity (Bastian 1984). A skin reaction triggered by exposure to sunlight was described in another study (Levine 1983). Research regarding the mechanism involved in photosensitivity to 4,4′-methylenedianiline would be helpful. Examination of

subjects occupationally exposed (presumably long-term exposure) revealed that 4,4′-methylenedianiline can be absorbed through the lungs and the skin and that the parent compound and metabolites can be excreted in the urine (Cocker et al. 1986a, 1994; Schiitze et al. 1995). A limited number of oral studies in animals showed that the liver, thyroid, and perhaps the kidney are targets for 4,4′-methylenedianiline toxicity (Deichmann et al. 1978; Lamb et al. 1986; NTP 1983). One of these studies, Deichmann et al. (1978), was conducted in dogs and the results suggested that dogs may be a particularly sensitive species for liver and kidney effects. However, this study was not well designed (only 9 dogs were tested and no concurrent controls were used), and therefore, the results are inconclusive. The inadequacies of this study precluded derivation of a chronic oral MRL. A well conducted chronic oral study in dogs could remove the uncertainty regarding dogs as very sensitive species for 4,4′-methylenedianiline toxicity. A comprehensive clinical evaluation of individuals exposed to low levels of 4,4′-methylenedianiline for many years in the workplace may provide evidence on less recognizable subtle effects of 4,4′-methylenedianiline. Neither immunocompetence nor reproductive function has been examined in animals after chronic exposure to 4,4′-methylenedianiline by any route of exposure. Such studies after low-level chronic exposure by the oral and dermal routes would be of value to determine whether exposures via these routes could cause toxicity in populations living near hazardous waste sites, or in those exposed in industries where this chemical is used.

Two-year cancer bioassays have been performed following oral and dermal exposure. In a well designed 2-year drinking water bioassay, there was clear evidence of carcinogenicity in rats and mice (Lamb et al. 1986; NTP 1983). An oral bioassay in dogs showed no evidence of carcinogenicity, but, as previously mentioned, the study was poorly designed and therefore, the results are inconclusive (Deichmamr et al. 1978). A dermal bioassay in mice found 4,4′-methylenedianiline to be a liver carcinogen, but it appears that the strain of mice used may have been particularly susceptible to liver tumors; therefore, the results are also inconclusive (Holland et al. 1987). A follow-up study on a group of individuals who had accidentally ingested 4,4′-methylenedianiline and contracted toxic hepatitis did not find increased incidences of cancer (Hall et al. 1992). A similar conclusion was reached in a small group of power generation workers exposed to an epoxy resin containing 4,4′-methylenedianiline (Selden et al. 1992). Two additional occupational studies found inconclusive evidence of bladder cancer in workers who were also exposed to other chemicals (Cragle et al. 1992; Liss and Guirguis 1994). Follow-up and other epidemiological studies are needed to adequately assess the carcinogenicity of 4,4′-methylenedianiline in humans.

Genotoxicity. The only information in humans was provided by Ho et al. (1979), who reported that incubation of human leukocytes with 4,4′-methylenedianiline did not increase the incidence of sister chromatid exchanges. Existing genotoxicity studies indicate that 4,4′-methylenedianiline was mutagenic in *Salmonella* (Andersen et al. 1980; Cocker et al. 1986b; LaVoie et al. 1979; McCarthy et al. 1982; Parodi et al. 1981; Rao et al. 1982; Tanaka et al. 1985) with metabolic activation. 4,4′-Methylenedianiline also induced DNA damage in rat hepatocytes (Mirsalis et al. 1983; Mori et al. 1988) *in vitro* and formed adducts with rat liver DNA (Endo and Hara 1991; Schiitze et al. 1996; Vock et al. 1996). Cytogenic analysis of human populations exposed to 4,4′-methylenedianiline in occupational settings would provide an opportunity to assess the genotoxic potential of this chemical in humans.

Reproductive Toxicity. No studies were located regarding reproductive effects in humans after exposure to 4,4′-methylenedianiline by any route. A small number of studies provided information on the gross and histopathological appearance of reproductive organs in animals after acute (Tullner 1960), intermediate (NTP 1983), and chronic (Deichmann et al. 1978; NTP 1983) oral exposure to 4,4′-methylenedianiline. Hypertrophy of the uterus in rats was described in an acute study (Tullner 1960), and endometrial hyperplasia was reported in dogs after chronic treatment with relatively low 4,4′-methylenedianiline doses (Deichmann et al. 1978). However, the latter study has severe limitations (small number of animals and no concurrent controls) and the results should be interpreted with caution. A single acute dermal study found no alterations in the testes and epididymis from rabbits after treatment with 4,4′-methylenedianiline (DuPont 1975). Since none of these studies examined reproductive function, additional studies are necessary to adequately evaluate this parameter. A multi-generational study would provide valuable information. Examination of clinical records from women exposed for prolonged periods of time to low levels of 4,4′-methylenedianiline at work may provide information on reproductive outcome for this population. Oral and dermal studies in animals would provide information on susceptibility by these routes. The dermal route is of particular concern in individuals exposed to 4,4′-methylenedianiline in the workplace.

Developmental Toxicity. No studies were located regarding developmental effects in humans after exposure to 4,4′-methylenedianiline by any route. A single study in rats showed that exposure to 4,4′-methylenedianiline during, gestation may result in adverse developmental effects (Bourdelat et al. 1983). The study, however, was of limited scope and was not well conducted. Further well-designed studies in animals exposed during pregnancy would provide information on the potential effects of

4,4′-methylenedianiline on the developing organism. Studies by the dermal route are of particular interest since this route is of concern in occupational settings and to people living near hazardous waste sites where 4,4′-methylenedianiline might be found.

Immunotoxicity. Information in humans indicates that dermal contact with 4,4′-methylenedianiline may trigger allergic dermal reactions in some individuals (Emmett 1976; McGill and Motto 1974; Van Joost et al. 1987). In one case, a reaction was triggered by exposure to sunlight (Levine 1983). Animal studies have provided limited information. An acute oral study found histopathological alterations in the thymus from rats (Kanz et al. 1992). Intermediate oral studies found no histopathological alterations in organs of the lymphoreticular system from rats and mice (NTP 1983). Similar findings were reported in chronic oral studies in rats and mice (NTP 1983). The NTP (1983) study did not evaluate blood components. A chronic oral study in dogs reported spleen effects at a relatively low dose (Deichmann et al. 1978); however, because of study limitations, the results must be interpreted with caution. Two acute dermal studies provided information on spleen weight in mice (Holland et al. 1987) and on histopathology of spleen and thymus in rabbits (DuPont 1975). None of the studies in animals examined immunocompetence. Studies that examine antibody levels and responses to bacterial and viral infections after exposure to 4,4′-methylenedianiline would provide valuable information on the immune system. Some immune parameters (e.g., serum immunoglobulin levels, response to mitogen stimulation) have been found to be quite sensitive to chemical insult. Also, evaluation of morbidity among individuals exposed to 4,4′-methylenedianiline in the workplace may provide important indirect evidence regarding their immune status.

Neurotoxicity. No studies were located regarding neurotoxicity in humans after exposure to 4,4′-methylenedianiline by any route. An acute dermal study in rabbits (DuPont 1975) and intermediate and chronic oral studies in rats and mice (Lamb et al. 1986; NTP 1983) found no gross or histopathological lesions in tissues from the peripheral and central nervous system. However, more sensitive neurological end points have not been examined. Laboratory animal studies that focus on subtle neurological effects following acute, intermediate, or chronic exposure via oral and dermal routes would help estimate potential neurotoxic effects in humans living near hazardous waste sites and in workers who might be exposed in certain occupational settings. Furthermore, evaluation of neurological end points in offspring from animals exposed during gestation would provide information that may be relevant to children of pregnant women exposed to 4,4′-methylenedianiline in the workplace.

Epidemiological and Human Dosimetry Studies. A retrospective study investigated the relationship between deaths caused by neoplastic and non-neoplastic diseases and accidental ingestion of 4,4′-methylenedianiline in a cohort originally composed of 84 subjects (Hall et al. 1992). The study period covered approximately 20 years after the accident occurred. The results showed no obvious link between ingestion of 4,4′-methylenedianiline and any particular cancer or non-neoplastic disease. Another study conducted a retrospective assessment of exposure to 4,4′-methylenedianiline and cancer morbidity in power generation workers in Sweden (Selden et al. 1992). An overall standardized cancer incidence ratio of 0.52 indicated no increased incidence of cancer due to exposure to 4,4′-methylenedianiline, but several study limitations were identified (small size and young age of the cohort, and short follow-up period). These limitations should be addressed in additional wellconducted epidemiology studies in occupational settings. This information would provide valuable data on possible adverse effects in humans. There is evidence that 4,4′-methylenedianiline may be released from certain medical devices made of polyurethane such as plasma separators and artificial dialyzers (Shintani 1991). This suggests a potential health risk for uremic patients or patients who receive frequent blood transfusions. Because of the known hepatic effects of 4,4′-methylenedianiline, liver tests should be periodically conducted in these patients as well as in occupationally exposed individuals for early detection of liver toxicity.

Biomarkers of Exposure and Effect.

Exposure. Exposure to 4,4′-methylenedianiline may be assessed by determining levels of 4,4′-methylenedianiline or N-acetylated metabolites in urine (Cocker et al. 1986a, 1994; Dalene et al. 1995; Robert et al. 1995; Schfitze et al. 1995). However, no quantitative estimates of exposure have been provided. In a study of 411 individuals occupationally exposed, 42% of urine samples examined had no detectable 4,4′-methylenedianiline (Cocker et al. 1994). In another study of 111 workers exposed to 4,4′-methylenedianiline, 81% of over 300 urine samples had concentrations of 4,4′-methylenedianiline below 5 nmol/mmol creatinine, which was below the detection limit of 50 nmol/L (Cocker et al. 1986a). A preferred method for assessing exposure may be bpmeasuring hemoglobin adducts. Studies in rats have shown that 4,4′-methylenedianiline can form adducts with hemoglobin which are stable *in vivo* and persist for the life span of the erythrocyte-120 days in humans (Bailey et al. 1990; Farmer and Bailey 1989). Similar findings have recently been reported in humans (Schiitze et al. 1995). 4,4′-Methylenedianiline can be released from the adduct and quantitated. The advantage of this method is that it provides information that is not restricted to recent

exposure. Preliminary results in rats showed that a dose-response can be established. Further studies on this subject would provide valuable information that could lead to early detection of 4,4′-methylenedianiline exposure.

Effect. There are no specific biomarkers of effects for 4,4′-methylenedianiline. Many different classes of chemicals are liver toxicants or may cause dermal sensitization. Further studies to identify specific biomarkers of effects of 4,4′-methylenedianiline would facilitate medical surveillance leading to early detection of potentially adverse health effects and possible treatment.

Absorption, Distribution, Metabolism, and Excretion. There is no quantitative information on the rates of absorption of 4,4′-methylenedianiline in humans following inhalation or oral exposure. A study in volunteers showed that a maximum of approximately 28% of a dose of 4,4′-methylenedianiline applied dermally for one hour was absorbed (Brunmark et al. 1995). Limited data from workers exposed to 4,4′-methylenedianiline su,, Ooested that absorption by inhalation is faster than dermal absorption (Cocker et al. 1994). There is also no information on absorption rates in animals following inhalation or oral exposure. Dermal data are available in rats, guinea pigs, and monkeys (El-Hawari et al. 1986). Obtaining additional quantitative data in animals via inhalation and oral routes, and using different vehicles, would be helpful for estimating absorption in humans.

There are no distribution data in humans. Data on distribution via the inhalation and dermal routes for animals were not located. There is limited information describing distribution following acute dermal exposure to 4,4′-methylenedianiline in animals. Studies indicate that it is distributed among all tissues (El-Hawari et al. 1986). Additional studies regarding repeated dermal exposure would help elucidate the distribution pattern of 4,4′-methylenedianiline. The dermal route appears to be the most likely route of exposure near hazardous waste sites. Dermal contact is also the most likely route of exposure in manufacturing and process industries.

No information was located regarding metabolism of 4,4′-methylenedianiline in humans-following inhalation or oral exposure. Data from humans exposed primarily by dermal contact showed that 4,4′-methylenedianiline can be acetylated *in vivo* to form N-acetylmethylenedianiline (Cocker et al. 1986a; Robert et al. 1995) and to a small extent, N,N’-diacetylmethylenedianiline (Robert et al. 1995). Acetylation has been demonstrated in rats exposed orally (Tanaka et al. 1985). No information was located regarding metabolism in animals exposed by inhalation or dermally. Information on

metabolism after dermal exposure in animals would be useful because the potential exists for exposure to occur in humans via this route.

Workers and volunteers exposed to 4,4′-methylenedianiline by inhalation or by skin contact excreted 4,4′-methylenedianiline and/or acetylated metabolites in the urine (Brunmark et al. 1995; Cocker et al. 1986a, 1994; Dalene et al. 1995). There is no information regarding excretion in humans after oral exposure. Acute dermal studies in animals showed that urinary excretion is a major excretory pathway, but fecal excretion also occurs (El-Hawari et al. 1986). Only one study was located that provided excretion data in animals following oral exposure (Tanaka et al. 1985); no inhalation data were located. Further examination of excretion patterns in animals following repeated dermal exposures would provide valuable information relevant to humans exposed continuously in the workplace.

Comparative Toxicokinetics. There is insufficient information to determine possible differences or similarities in toxicokinetic patterns for 4,4′-methylenedianiline between humans and animals. The limited data suggest that 4,4′-methylenedianiline can be acetylated by humans and animals, and that the resulting metabolite is excreted in the urine. Studies have also demonstrated that the liver is a target for 4,4′-methylenedianiline toxicity in humans and the animal species tested. Acute dermal studies in animals showed some qualitative differences in excretory patterns between species. The urine was the main excretory route in rats and monkeys, whereas guinea pigs excreted similar amounts of 4,4′-methylenedianiline (or metabolites) in urine and feces (El-Hawari et al. 1986). Physiologically based pharmacokinetic models for 4,4′-methylenedianiline have not been developed. Further studies are necessary to determine which species might be a suitable animal model.

Methods for Reducing Toxic Effects. The mechanism by which 4,4′-methylenedianiline enters the bloodstream in humans is not known, and there are no established methods for reducing absorption after inhalation and oral exposure other than minimizing exposure. A study of dermal absorption through human skin *in vitro* provided information on decontamination procedures that could potentially be used *in vivo* (Hewitt et al. 1995). Further studies of decontamination strategies by using different washing solutions would be valuable. Animal studies using gastrointestinal sorbents such as activated carbon and resins, which can bind amines, might give insight into additional methods for reducing systemic absorption of this chemical. Suggested methods for treating the effects of acute exposure to 4,4′-methylenedianiline are generally supportive. Toxic hepatitis that developed after exposure to

4,4´-methylenedianiline has been treated conventionally. There are no established methods for reducing body burdens in humans. It is assumed that metabolism of 4,4´-methylenedianiline leads to the formation of highly reactive and potentially toxic derivatives. Thus, additional studies examining the feasibility of favoring metabolic pathways leading to the formation of nontoxic metabolites (N-acetylation pathway) would be valuable.

2.10.3 Ongoing Studies

Dr. Mary Kanz from the Department of Pathology, University of Texas at Galveston, is conducting research aimed to understand how and why 4,4´-methylenedianiline causes early, selective injury to bile duct cells. She is focusing the research on 4,4´-methylenedianiline metabolites excreted in bile and their capacity to damage biliary epithelial cells (FEDRIP 1997).