3. HEALTH EFFECTS

3.1 INTRODUCTION

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

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

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

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

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of TDI and MDI are indicated in Tables 3-2 and 3-5 and Figures 3-2, and 3-3.

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

3.2.1 Inhalation Exposure

The highest NOAEL values and all LOAEL values from each reliable study for each end point in each species and duration category are recorded in Tables 3-1 and 3-2 and plotted in Figures 3-1 and 3-2 for TDI and MDI, respectively.

3.2.1.1 Death

Available literature did not include human studies evaluating lethality after inhalation exposure to TDI or MDI.

TDI. Acute-duration exposure to commercial-grade TDI at concentrations up to 1.0 ppm did not result in any deaths when groups of eight pregnant CD rats were exposed during GDs 6–15 in a dose-range finding study (Tyl et al. 1999a). Likewise, exposure concentrations up to 0.5 ppm did not result in maternal deaths in the main developmental toxicity study (Tyl et al. 1999a) or in F0 or F1 parental animals in a 2-generation reproductive toxicity study using rats (Tyl et al. 1999b). Chronic (2-year) exposure to 0.15 ppm production-grade TDI (80:20 mix of 2,4- and 2,6-TDI) did not affect survival rates of Sprague-Dawley rats (Loeser et al. 1983). In CD-1 mice, a significantly increased rate of mortality was seen with

Table 3-1. Levels of Significant Exposure to Toluene Diisocyanate - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Systemic								
1	Human	6 hr	Resp		0.005[b] (slight decrease in specific airway conductance)		Vandenplas et al. 1999 TDI, not specified	
2	Rat (Wistar)	4 hours/day 5 days	Resp		0.41 F (hypersensitivity symptoms, central airway goblet cell metaplasia, central and peripheral airway eosinophil infiltration)		Kouadio et al. 2014 2,4-TDI	
3	Rat (CD)	6 hr/d Gd 6-15	Resp		0.02 F (red nasal discharge in 5/21 dams)		Tyl et al. 1999a 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
			Hepatic	0.5 F				
			Bd Wt			0.5 F (45% decrease in maternal body weight gain during exposure)		
4	Rat (CD)	6 hr/d Gd 6-15	Resp		1 F (maternal nasal discharge and labored respiration; blood gas changes indicative of respiratory acidosis)		Tyl et al. 1999a 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
			Bd Wt			1 F (27% decrease in body weight)		

Table 3-1. Levels of Significant Exposure to Toluene Diisocyanate - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
5	Mouse (BALB/c)	45, 90, 180, or 360 min/d 3 d	Resp		1 M (severe nasal lesions and slight laryngeal lesions)		Arts et al. 2008 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
6	Mouse (Swiss-Webster)	6 hr/d 5 d	Resp		0.4 M (moderate to severe nasal lesions)		Buckley et al. 1984 TDI, not specified	
7	Mouse (C57BL/6N)	4 hr/d 12 d	Resp		0.05 F (cellular inflammation and hyperplasia in anterior nasal cavity)		Johnson et al. 2007 TDI, not specified	
8	Mouse (C57BL/6N)	2 hr	Resp		0.5 F (nasal and lung inflammation, airway hyperresponsiveness)		Matheson et al. 2005 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
9	Mouse (Swiss-Webster)	3 hr/d 5 d	Resp	0.031 M 0.018 M	0.25 M (histological damage to nasal respiratory epithelium) 0.023 M (decreased respiratory rate)		Sangha and Alarie 1979 2,4-TDI	

Table 3-1. Levels of Significant Exposure to Toluene Diisocyanate - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
10	Mouse (Swiss)	6 hr/d 4 d	Resp		0.07 M (moderate rhinitis with metaplasia and necrosis in the nasal respiratory epithelium)		Zissu 1995 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
11	Gn Pig (Hartley)	3 hr/d 5 d	Resp	0.02 F	0.2 F (pulmonary response to TDI challenge)		Aoyama et al. 1994 2,4/2,6-TDI	
12	Gn Pig (Dunkin-Hartle	1 hr	Resp		3 F (airway hyperresponsiveness)		Gagnaire et al. 1996 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
13	Gn Pig (Dunkin-Hartle	continuously 48 hr	Resp		0.1 F (airway hyperresponsiveness)		Gagnaire et al. 1996 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
14	Gn Pig (Dunkin-Hartle	continuously 1 wk	Resp		0.05 F (airway hyperresponsiveness)		Gagnaire et al. 1996 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
15	Gn Pig (Hartley)	1 hr	Resp		2 M (airway hyperresponsiveness, tracheal epithelial damage, acute airway inflammation)		Gordon et al. 1985 TDI, not specified	

Table 3-1. Levels of Significant Exposure to Toluene Diisocyanate - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
16	Gn Pig (Dunkin Hartley)	6 hr/d 5 d	Resp	0.005 F	0.01 F (increased airway responsiveness)		Marek et al. 1999 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
17	Gn Pig (English smooth haired)	3 hr/d 5 d	Resp		1.4 F (diminished response to CO2, pulmonary hypersensitivity, interstitial inflammation, pleural thickening and goblet cell hyperplasia in the lungs)		Wong et al. 1985 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
Developmental								
18	Rat (CD)	6 hr/d Gd 6-15		0.1	0.5 (increased incidence of litters with poorly ossified cervical centrum no. 5)		Tyl et al. 1999a 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
INTERMEDIATE EXPOSURE								
Systemic								
19	Rat (CD)	2-generation, 19 wk 5 or 7 d/wk 6 hr/d	Resp		0.02 (rhinitis in F1 parental animals)		Tyl et al. 1999b 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
			Hepatic	0.3				
			Renal	0.3 F				
			Endocr	0.3				
			Bd Wt	0.3				

Table 3-1. Levels of Significant Exposure to Toluene Diisocyanate - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
20	Mouse (C57BL/6N)	4 hr/d 5 d/wk 6 wk	Resp		0.02 F (nasal and lung inflammation, airway hyperresponsiveness)		Matheson et al. 2005 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
21	Mouse (Swiss)	6 hr/d 5 d/wk 9 or 14 exposures	Resp		0.07 M (severe rhinitis with metaplasia and necrosis in the nasal respiratory epithelium)		Zissu 1995 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
22	Gn Pig (English smooth haired)	6 hr/d 4 d/wk 14 wk	Resp	0.2 F			Wong et al. 1985 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
Developmental								
23	Rat (CD)	2-generation, 19 wk 5 or 7 d/wk 6 hr/d			0.08 (9% lower body weight gain of F2 pups during lactation)		Tyl et al. 1999b 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
CHRONIC EXPOSURE								
Death								
24	Mouse (CD-1)	104 wk 5 d/wk 6 hr/d				0.05 F (significantly increased mortality, 77% vs 60% in controls)	Loeser 1983 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
Systemic								
25	Human	occupational exposure	Resp	0.0023			Bodner et al. 2001 2,4/2,6-TDI	

Table 3-1. Levels of Significant Exposure to Toluene Diisocyanate - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
26	Human	occupational exposure	Resp		0.015	(respiratory symptoms)	Butcher et al. 1977 2,4/2,6-TDI	
27	Human	occupational exposure	Resp		0.0012[c]	(decline in FEV1 in naive subjects)	Clark et al. 1998 2,4/2,6-TDI	
28	Human	occupational exposure	Resp		0.00105	(increased reporting of wheezing)	Clark et al. 2003 2,4/2,6-TDI	
29	Human	occupational exposure	Resp		0.0082	(decreased lung function)	Omae et al. 1992 2,4/2,6-TDI	
30	Human	occupational exposure	Resp	0.0042			Ott et al. 2000 2,4/2,6-TDI	
31	Human	occupational exposure	Resp		0.0035	(decreased lung function)	Wegman et al. 1977, 1982 2,4/2,6-TDI	

Table 3-1. Levels of Significant Exposure to Toluene Diisocyanate - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
32	Rat (Sprague-Dawley)	108-110 wk 5 d/wk 6 hr/d	Cardio	0.15			Loeser 1983 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
			Gastro	0.15				
			Hemato	0.15				
			Musc/skel	0.15				
			Hepatic	0.15				
			Renal	0.15				
			Endocr	0.15				
			Bd Wt	0.15				
33	Mouse (CD-1)	104 wk 5 d/wk 6 hr/d	Resp			0.05 (chronic or necrotic rhinitis)	Loeser 1983 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
			Cardio	0.15				
			Gastro	0.15				
			Hemato	0.15				
			Musc/skel	0.15				
			Hepatic	0.15				
			Renal	0.15				
			Endocr	0.15				

Table 3-1. Levels of Significant Exposure to Toluene Diisocyanate - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
			Bd Wt		0.15 (significantly reduced weight gain)			

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

b Used to derive an acute-duration inhalation MRL of 0.00001 ppm. The LOAEL was adjusted for intermittent exposure (6 hours/day) and divided by an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability).

c Used to derive a chronic-duration inhalation MRL of 0.000003 ppm. The mean daily exposure level was adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = Female; FEV1 = forced expiratory volume in 1 second; Gastro = gastrointestinal; Gd = gestational day; Gn Pig = guinea pig; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Figure 3-1. Levels of Significant Exposure to Toluene Diisocyanate - Inhalation

Acute (≤14 days)

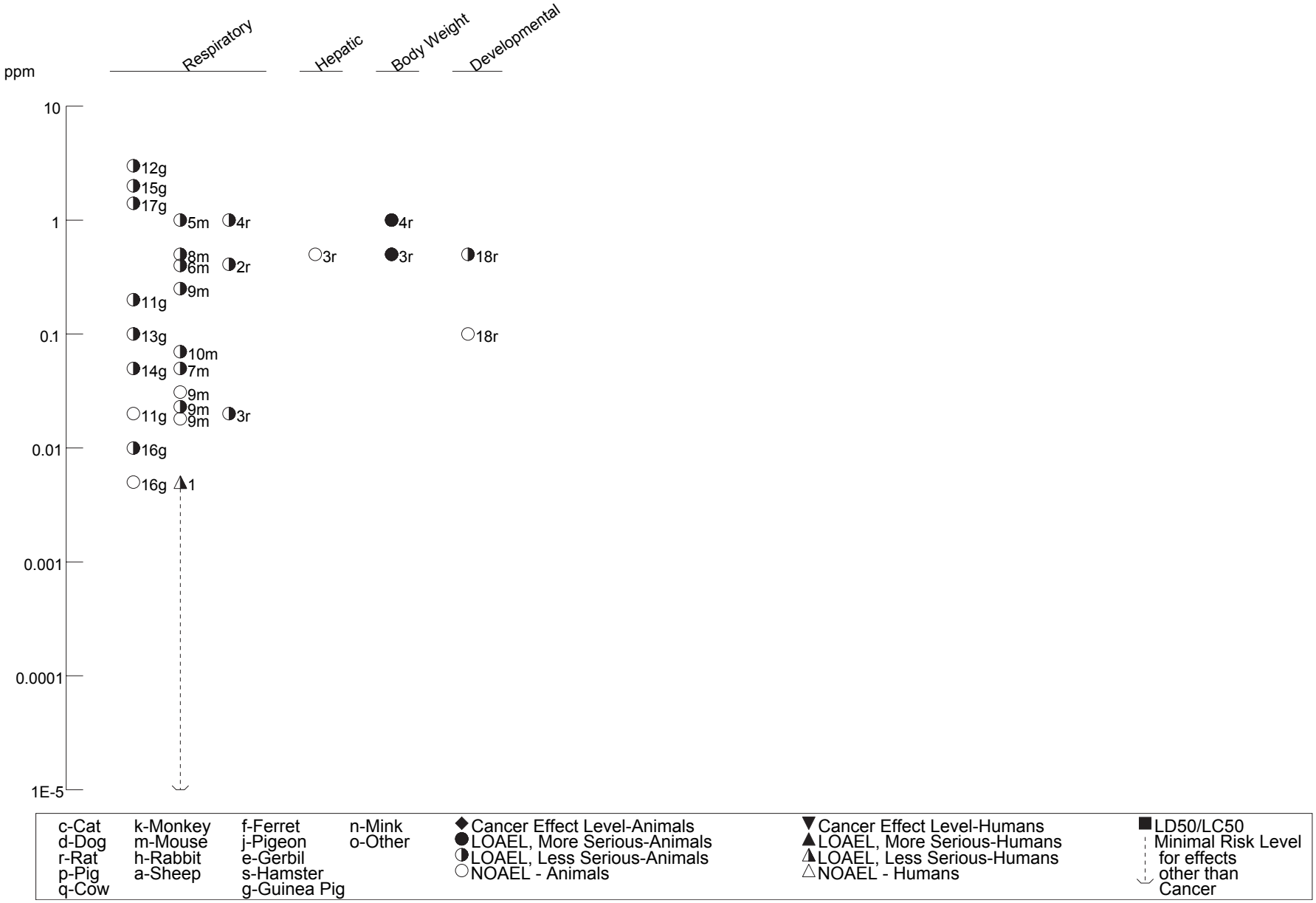


Figure 3-1. Levels of Significant Exposure to Toluene Diisocyanate - Inhalation *(Continued)*

Intermediate (15-364 days)

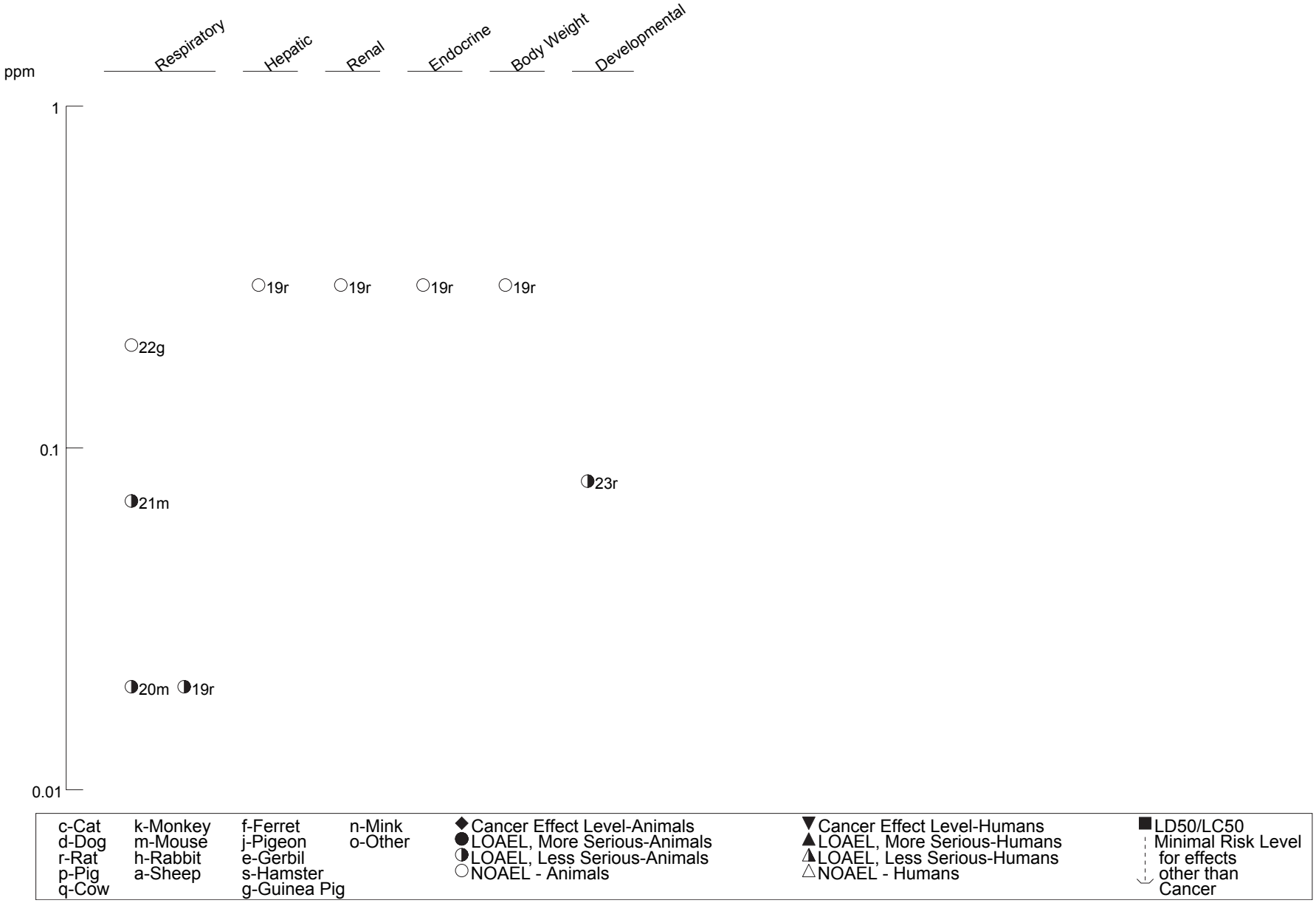


Figure 3-1. Levels of Significant Exposure to Toluene Diisocyanate - Inhalation *(Continued)*

Chronic (≥365 days)

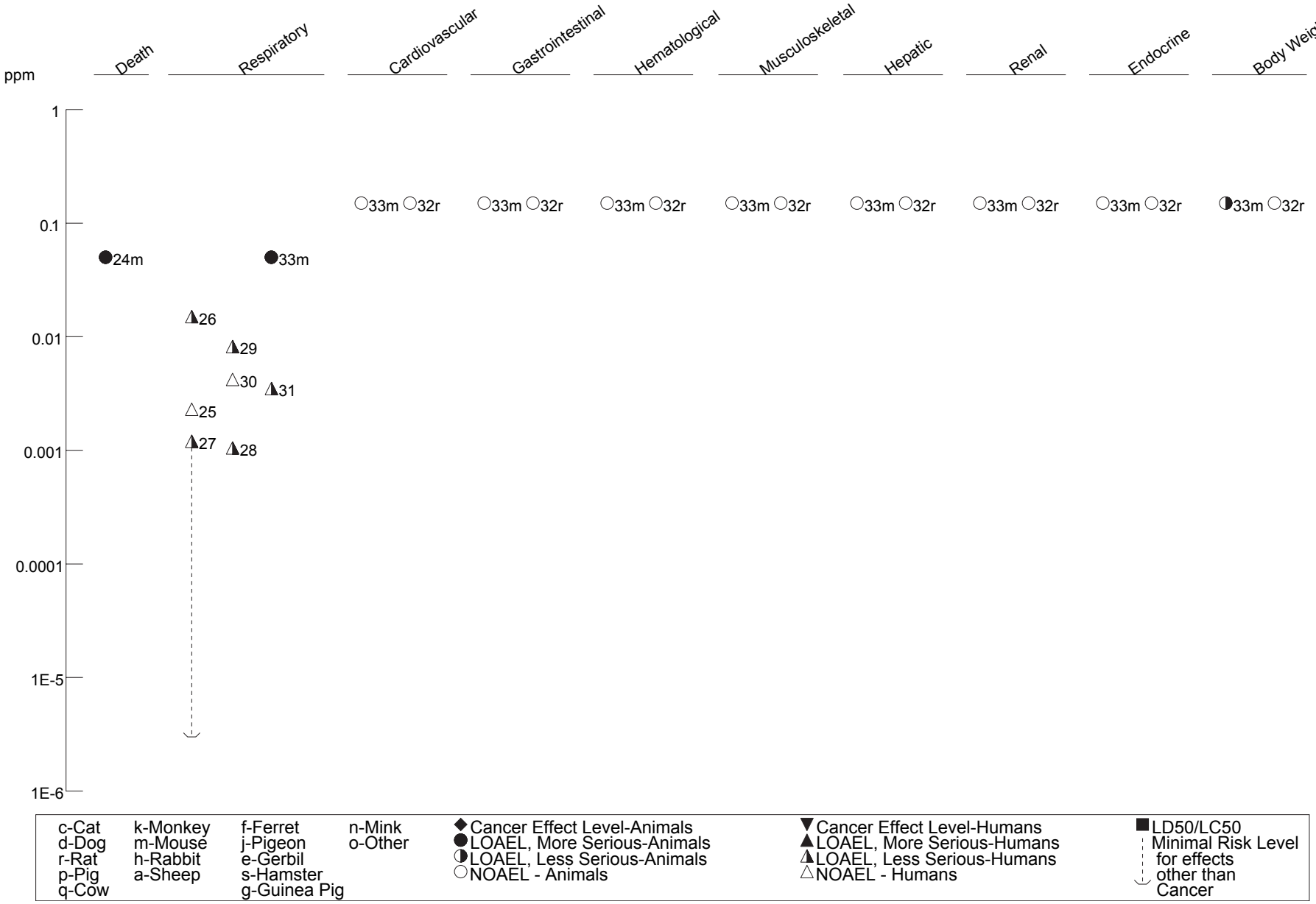


Table 3-2. Levels of Significant Exposure to Methylene Diphenyl Diisocyanate - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Wistar)	Gd 6-15 6 hr/d		9 F			Buschmann et al. 1996 4,4'-MDI	
Systemic								
2	Rat (Wistar)	Gd 6-15 6 hr/d	Resp		9 F (24% increase in relative lung weight)		Buschmann et al. 1996 4,4'-MDI	
			Bd Wt	9 F				
3	Gn Pig (Dunkin Hartley)	6 hr/d 5 d	Resp	0.0005 F	0.001 F (increased airway responsiveness)		Marek et al. 1999 4,4'-MDI	
Developmental								
4	Rat (Wistar)	Gd 6-15 6 hr/d			9 (increased litter incidence of asymmetric sternebrae)		Buschmann et al. 1996 4,4'-MDI	
INTERMEDIATE EXPOSURE								
Systemic								
5	Gn Pig (Dunkin Hartley)	6 hr/d 5 d/wk 4 wk	Resp		0.001 F (increased airway responsiveness)		Marek et al. 1999 4,4'-MDI	
CHRONIC EXPOSURE								
Death								
6	Rat (Wistar)	24 mo 5 d/wk 6 hr/d		6			Reuzel et al. 1994 Polymeric MDI, aerosolized	

Table 3-2. Levels of Significant Exposure to Methylene Diphenyl Diisocyanate - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
Systemic								
7	Human	occupational exposure	Resp	0.0005			Sulotto et al. 1990 4,4'-MDI	
8	Rat (Wistar)	24 mo 5 d/wk 6 hr/d	Resp	0.2[b]	1 (minimal to mild pulmonary fibrosis and macrophage accumulation; alveolar duct epithelialization; basal cell and Bowman's gland hyperplasia in the nasal cavity)		Reuzel et al. 1994 Polymeric MDI, aerosolized	
			Cardio	6				
			Hemato	6				
			Hepatic	6				
			Renal	6				
			Endocr	6				
			Bd Wt	6				
Immuno/ Lymphoret								
9	Rat (Wistar)	24 mo 5 d/wk 6 hr/d		0.2 M	1 M (minimal to mild macrophage accumulation in mediastinal lymph nodes)		Reuzel et al. 1994 Polymeric MDI, aerosolized	
Neurological								
10	Rat (Wistar)	24 mo 5 d/wk 6 hr/d		6			Reuzel et al. 1994 Polymeric MDI, aerosolized	

Table 3-2. Levels of Significant Exposure to Methylene Diphenyl Diisocyanate - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
Reproductive								
11	Rat (Wistar)	24 mo 5 d/wk 6 hr/d		6 M			Reuzel et al. 1994 Polymeric MDI, aerosolized	
Cancer								
12	Rat (Wistar)	24 mo 5 d/wk 6 hr/d				6 M (CEL: lung adenomas and adenocarcinomas)	Reuzel et al. 1994 Polymeric MDI, aerosolized	

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

b Used to derive a chronic-duration inhalation MRL of 0.001 mg/m3 for polymeric MDI based on a BMDL of 0.48 mg/m3. The BMDL was adjusted for intermittent exposure and multiplied by the regional deposited dose ratio for extrathoracic effects to calculate the human equivalent concentration (HEC). The BMDL(HEC) of 0.039 mg/m3 was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; F = female; Gd = gestational day; Gn Pig = guinea pig; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = weeks(s)

Figure 3-2. Levels of Significant Exposure to Methylene Diphenyl Diisocyanate - Inhalation

Acute (≤14 days)

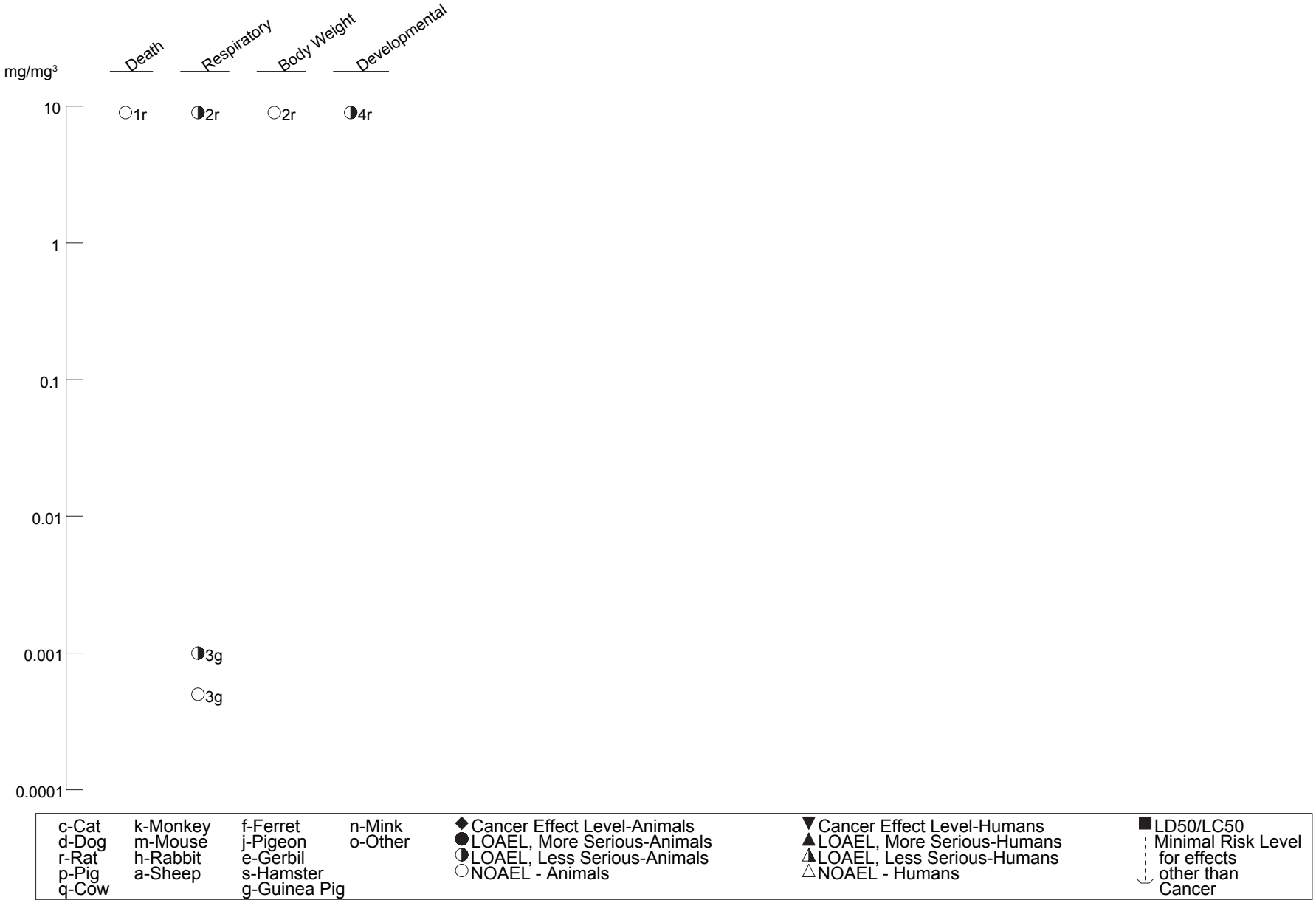
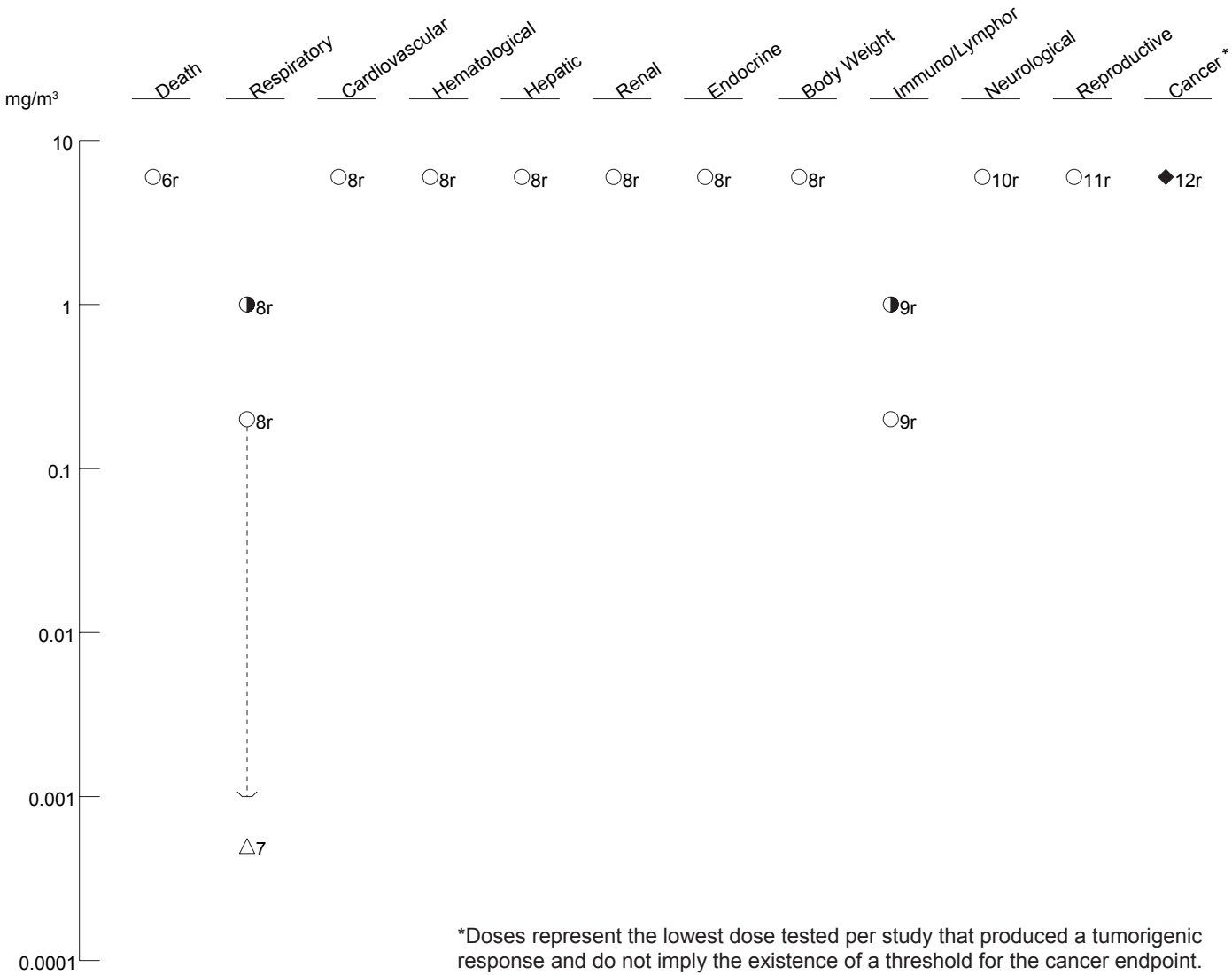


Figure 3-2. Levels of Significant Exposure to Methylene Diphenyl Diisocyanate - Inhalation *(Continued)*

Intermediate (15-364 days)

Figure 3-2. Levels of Significant Exposure to Methylene Diphenyl Diisocyanate - Inhalation *(Continued)*

Chronic (≥365 days)



chronic exposure to ≥0.05 ppm production-grade TDI; deaths occurred in 60% of controls and in 77 and 74% of low- and high-exposure females, respectively (Loeser et a. 1983).

MDI. No deaths were observed in acute- or intermediate-duration studies in guinea pigs exposed up to 20 ppb 4,4'-MDI (Marek et al. 1999), in a developmental toxicity study in which rats were exposed to 9 mg/m^3 4,4'-MDI aerosol on GDs 6–15 (Buschmann et al. 1996), or in a chronic-duration study in which rats were exposed to 6.0 mg/m^3 for 2 years (Reuzel et al. 1994).

3.2.1.2 Systemic Effects

Respiratory Effects.

TDI. A large number of epidemiology and experimental studies have examined the toxicity of TDI to the respiratory tract. Data from a limited number of acute-duration studies identify respiratory irritation as the primary effect at low concentrations and severe respiratory symptoms and possibly asthma occurring after exposure to high concentrations. As reviewed by Ott et al. (2003), a 30-minute exposure to TDI resulted in the following effects in healthy subjects: ocular irritation at 0.050 ppm, nasal irritation at 0.080 ppm, eye, nose, and throat irritation, which was considered tolerable, at 0.100 ppm; tearing and burning in the throat at 0.50 ppm, and nasal discharge and severe cough after several minutes of exposure to 1.3 ppm. Another study reviewed by Ott et al. (2003) reported chest tightness, cough, and burning of the throat in asthmatics (asthma was not due to occupational exposure to TDI) exposed to 0.01 or 0.02 ppm TDI for 1 hour. By comparison, another study reported a mild cough in 1/10 healthy subjects exposed to 0.02 ppm TDI for 2 hours. A longer study in healthy subjects exposed to 0.005 ppm TDI for 6 hours followed by a 20-minute exposure to 0.02 ppm did not result in respiratory symptoms (Vandenplas et al. 1999). However, slight, but statistically significant, decreases in specific airway conductance (sG_{AW}) and MEF at 25% of FVC ($MEF_{25\%}$) were observed. The decreases in sG_{AW} and $MEF_{25\%}$ started within the first 60 minutes of exposure. Another study (Chester et al. 1979) did not find alterations in specific airway resistance (sR_{AW}) in healthy or asthmatic (not TDI-induced) subjects exposed to 0.02 ppm TDI for 20 minutes.

The primary respiratory effects observed following longer-term occupational exposure are TDI-induced bronchial asthma, asthma-like respiratory symptoms, and a decline in pulmonary function. TDI-induced asthma is a type of occupational asthma that can be characterized as bronchial inflammation and/or airway hyperresponsiveness. Respiratory symptoms often reported in workers with TDI-induced asthma

include wheezing, dyspnea, coughing, and chest tightness, which often persist after TDI exposure has ceased (Mapp et al. 1988; Moller et al. 1986; Moscato et al. 1991; Padoan et al. 2003; Paggiaro et al. 1984). Individuals with TDI-induced asthma are considered to be sensitized to TDI, in that brief exposures to nonirritating concentrations can result in a worsening of symptoms and a decline in lung function. TDI exposure can result in four types of asthmatic reactions in sensitized individuals: an immediate response, a late response, a dual response (individuals having immediate and late responses), or an irregular response. Two studies, each examining about 100 workers suspected of having TDI-induced asthma based on respiratory symptoms (e.g., wheezing, dyspnea, chest tightness, or dry cough), found that more workers (41 or 44% of responders) had a late response to a TDI challenge (0.011 ppm for 30 minutes or 0.015–0.025 ppm for 10–15 minutes) than had a dual response (35 or 41%) or immediate response (28 or 21%) (Moscato et al. 1991; Paggiaro et al. 1986). A smaller scale study of 10 subjects reported that 8/10 workers had a late reaction to a TDI challenge (0.005–0.006 ppm) and 2/10 had a dual reaction (Saetta et al. 1995). Siracuse et al. (1978) reported a case of a worker who had recurrent nocturnal asthma as a result of TDI exposure. Although the cause of the different responses is not known, Paggiaro et al. (1986) noted that subjects who had a dual response to a TDI challenge had a significantly longer duration of symptoms before diagnosis than the immediate or late responders.

The prevalence of TDI-induced asthma among TDI workers has not been well established. A comparison of the prevalence of TDI-induced asthma across studies is difficult due to differences in the criteria used to define asthma. Some studies define asthma as removal from workplace or job due to respiratory symptoms, particularly wheezing and dyspnea, and others as a decrease in lung function following a TDI-challenge. Ott et al. (2003) calculated annual induction rates of TDI-induced asthma using data from a number of cross-sectional and longitudinal studies and found rates of approximately 5–6% prior to the 1970s and rates of <1% since the mid-1970s when TDI levels were typically maintained at ≤0.005 ppm and many of the cases were attributable to incidents involving exposure to TDI levels well above 0.02 ppm. In a study of 49 workers at a new TDI polyurethane foam manufacturing facility, new symptoms of asthma were observed in 7.1% of the workers after 6 months of exposure (Gui et al. 2014). The investigators noted that 90% of the air samples were less than the 0.0001 ppm detection limit, with a maximum exposure level of 0.01 ppm.

Inhalation challenge testing in which subjects with respiratory symptoms characteristic of asthma are exposed to a non-irritating concentration of TDI (typically ≤0.02 ppm) for a short period is often used to diagnose TDI-induced asthma. However, not all subjects have a positive reaction, such as a decline in FEV_1, to the challenge test (Banks et al. 1989; Burge 1982; Mapp et al. 1988; Moller et al. 1986; Moscato

et al. 1991; O'Brien et al. 1979). For example, a study of 58 workers reporting wheezing and dyspnea found that only 43% had a positive response in the TDI challenge test (Banks et al. 1989). Another study of 51 workers with respiratory symptoms found a positive reaction to the TDI challenge in 52% of the workers (Burge 1982).

The mechanism of TDI sensitization has not been elucidated; some investigators have suggested that immune mechanisms may be involved. Specific IgG antibodies to TDI-human serum albumin (HSA) conjugates (Park et al. 1999) or IgE antibodies to TDI-HSA (Baur and Fruhmann 1981; Cvitanovic et al. 1989; Park and Nahm 1996; Park et al. 1999; Pezzini et al. 1984; Sharifi et al. 2013) have been found in workers with TDI-induced asthma. However, TDI-HSA-specific IgG or IgE antibodies were typically found in less than half of the sensitized workers (16–57%).

A number of studies have tracked the prognosis of workers with probable TDI-induced asthma. Some recovery from respiratory symptoms and/or an absence of a response to a TDI challenge have been observed (Banks and Rando 1988; Banks et al. 1990; Lemiere et al. 1996; Mapp et al. 1998; Moller et al. 1986; Padoan et al. 2003; Paggiora et al. 1984, 1993; Park and Nahm 1997; Saetta et a. 1995). A small improvement in the prevalence of respiratory symptoms was observed 2 years after exposure termination in workers with verified TDI-induced asthma (Paggiaro et al. 1984). Dyspnea and wheezing were reported by 8/12 subjects, as compared to 12/12 subjects at the initial examination. In 16 asthmatic subjects who left the workplace, 56% did not respond to a TDI challenge administered 4 years after leaving the workforce (Paggiaro et al. 1993). Padoan et al. (2003) also reported a decline in the prevalence of respiratory symptoms of asthma and hyperresponsiveness to methacholine in subjects who ceased TDI exposure for an average of 11 years. However, 60% of the subjects removed from TDI exposure for >10 years still complained of asthmatic symptoms. Improvement in respiratory symptoms or response to a TDI challenge was not observed in workers who continued TDI exposure (Banks et al. 1990; Mapp et al. 1988; Padoan et al. 2003; Paggiaro et al. 1984). A study of 35 subjects with TDI-induced asthma monitored for 2 years after cessation of exposure found that 49% were no longer hyperresponsive to methacholine, 31% had significant improvements in the first year, and 20% did not show evidence of improvement. Subjects who recovered had a shorter duration of asthmatic symptoms before diagnosis, immediately ceased TDI exposure after diagnosis, had a milder degree of airway hyperresponsiveness, and had specific IgE antibodies to TDI-HSA (Park and Nahm 1997). A case report suggested that re-exposure to TDI may result in a reversal of the recovery (Banks and Rando 1988). Eleven years after a worker with asthma ceased TDI exposure, there were no respiratory symptoms and

no response when challenged with a subirritant concentration of TDI. However, within 5 months of returning to work, the subject developed asthma and had a positive response to a TDI challenge.

TDI-induced asthma is typically associated with acute exposure to very high concentrations or prolonged exposure to lower concentrations. Two studies have examined communities near a polyurethane foam manufacturing facility in Finland (Nuorteva et al. 1987) or TDI-emitting sources in North Carolina (Wilder et al. 2011). In the Finnish study of 3,153 adults living near the facility, asthma was diagnosed in 2.2% of the subjects, compared to 2.4% of the 1,029 subjects living in a referent area. In the subjects with asthma, IgE antibodies specific to TDI, MDI, or hexamethylene diisocyanate (HDI) were only found in one subject who was occupationally exposed. No differences in the prevalence of respiratory symptoms were found. Wilder et al. (2011) did not find a significant increase in the prevalence of asthma or asthma-like respiratory symptoms in the residents living near TDI sources compared to the referent communities. Of the 161 residents living near TDI sources, only 1 had IgG antibodies specific to TDI and none had IgE antibodies specific to TDI.

The primary effect in workers not sensitized to TDI is a decline in lung function. Ott et al. (2003) noted that the decline in lung function, particularly airflow limitations, may be due to increased airway wall thickness, subepithelial fibrosis, obstruction of airway lumen by exudate or mucus, and changes in elastic properties of airway walls or loss of the interdependence between airways and surrounding parenchyma. Longitudinal studies, summarized in Table 3-3, have examined possible changes in lung function in TDI workers over time and found mixed results based on reported 8-hour TWA TDI levels (Adams 1975; Bodner et al. 2001; Butcher et a. 1977; Clark et al. 1998, 2003; Diem et al. 1982; Jones et al. 1992; Omae et al. 1992; Ott et al. 2000; Peters et al. 1970; Wegman et al. 1977, 1982). Conflicting results may be due to differences in peak exposure levels, length of exposure, historical TDI exposure, or inclusion of subjects with possible TDI-induced asthma. Diem et al. (1982) conducted a 5-year study of a new TDI manufacturing facility and found a greater decline in FEV_1 and $FEF_{25–50\%}$ among workers with a cumulative TDI exposure of ≥0.0682 ppm-months and who never smoked. A greater decline in FEV_1 was also observed in smokers with high cumulative exposure; however, the mean annual decline was similar to expected values from cross-sectional studies of normal populations. Based on unpublished information from Janet Hughes, EPA (IRIS 2003) reported a mean 8-hour TWA TDI level in the high cumulative exposure, never-smoking group of 0.0019 ppm; the mean 8-hour TWA TDI level for the low cumulative exposure, never-smoking group was 0.0009 ppm. Clark et al. (1998) found a significant

Table 3-3. Summary of Occupational Exposure Studies Examining the Effects of TDI on Lung Function

Study	Exposure	Effect
Adams 1975 Longitudinal study 565 TDI workers at two TDI manufacturing facilities examined between 1961 and 1972	Exposure levels not reported; the percentage of TDI levels that exceeded 0.02 ppm was 58–67% in 1962–1964 (most readings between 0.05 and 0.1 ppm) in plant 1, 21% and 13% in 1965 in plants 1 and 2, and 1–4 and 2-8% in 1966–1970 in plants 1 and 2	No association between exposure and decline in FVC and FEV_1 levels
Bodner et al. 2001 Longitudinal study 305 TDI manufacturing workers employed for at least 3 consecutive months; referent group consisted of 581 workers in hydrocarbon departments at the same facility; workers were examined every 1–2 years for 26 years	Mean TDI level at the last study examination was 0.0023 ppm	No correlations between lung function (FEV_1 and FVC) and TDI exposure
Butcher et al. 1977 Longitudinal study 166 TDI manufacturing workers examined prior to working in TDI-related job and at 6-month intervals for a total of 2.5 years	The TWA TDI level was estimated to be 0.015 ppm based on area monitoring; a comparison between some area monitoring and personal monitoring data suggests that the area monitoring may overestimate the worker's exposure levels	No exposure-related decline in lung function
Clark et al. 1998 Longitudinal study 644 workers at 12 polyurethane foam manufacturing facilities and 136 referents examined over a 5-year period	The mean daily 8-hour TWA was 0.0012 ppm	No exposure-related decline in lung function; in a subset of 157 workers who entered the study after the first year, longitudinal analysis showed a significant decline in FEV_1 and FVC
Clark et al. 2003 Longitudinal study 251 polyurethane foam manufacturing workers (217 workers were part of the Clark et al. 1988 cohort)	Workers divided into three groups; mean 8-hour TWA TDI levels were 0.00105, 0.0006, and 0.00029 ppm	No effect of exposure on FEV_1 levels for the full cohort
Diem et al. 1982 Longitudinal study 114 TDI workers and 54 referents working at a new TDI manufacturing facility; workers divided in to low, medium, and high exposure groups and into high and low cumulative exposure groups; workers examined 9 times in 5-year period	Mean 8-hour TWA TDI levels were 0.0016, 0.0032, and 0.0068 ppm TDI levels in the low and high cumulative exposure groups were <0.0682 and ≥0.0682 ppm-months	Greater decline in FEV_1 and $FEF_{25–50\%}$ in never-smokers in the high cumulative exposure group, as compared to low cumulative exposure group; no effect on FEV_1 when TDI was expressed as a continuous variable

Table 3-3. Summary of Occupational Exposure Studies Examining the Effects of TDI on Lung Function

Study	Exposure	Effect
Gui et al. 2014 Longitudinal study 49 workers at a new TDI manufacturing facility	90% air samples had TDI levels below the detection limit of 0.0001 ppm	No significant alteration in FEV_1, FVC, or FEV_1/FVC; decline in FEV_1 levels of >15% in 9.1% workers after 12 months of exposure
Huang et al. 1991a Cross-sectional study 15 painters applying polyurethane varnish; included 7 workers with chronic bronchitis and 4 workers with dyspnea and wheezing	TDI levels ranged from 0.07 to 0.17 ppm	Decreased FVC, FEV_1, and FEV_1%
Huang et al. 1991b Cross-sectional study 48 workers at spraying polyurethane varnish at three facilities included workers at facilities A and B with chronic bronchitis (46.7 and 15%), dyspnea and wheezing (26.7 and 15%)	Mean TDI levels at facilities A, B, and C were 0.11, 0.043, and 0.015 ppm, respectively	Decreased FEV_1, and MMF at facilities A and B
Jones et al. 1992 Longitudinal study 386 workers at polyurethane foam manufacturing facility examined ≥1 time in a 2-year period; 227 examined at least 3 times and initial lung function testing performed on 294 workers	Mean TDI levels ranged from 0.00117 to 0.00447 ppm	No relationship between TDI exposure and decline in FVC or FEV_1 (excluded workers with TDI-induced asthma)
Olsen et al. 1989 Cross-sectional study 57 workers at TDI manufacturing facility and 89 referents	Exposure levels not reported; TDI levels did not exceed the permissible level of 0.02 ppm	No association between TDI exposure (current, highest career, or cumulative) and FEV_1 levels.
Omae et al. 1992 Longitudinal study 57 polyurethane foam manufacturing workers and 24 referents followed for 4 years	Mean TWA TDI levels in low and high exposure groups were 0.0001 and 0.0057 ppm High exposure group further divided into high-1 and high-2 groups; TWA TDI concentrations were 0.0082 ppm (maximum TWA TDI level of 0.02–0.03 ppm) and 0.0017 ppm (maximum TWA TDI level of 0.003–0.004 ppm)	Larger than expected annual losses of MMF, ratio of FEV_1%, $FEF_{25\%}$, and PEF in high-1 group; MMF, FEV_1%, and $PEF_{25\%}$ in the high-1 group were significantly higher than low exposure group
Ott et al. 2000 Longitudinal study 219 TDI manufacturing workers and 77 referents examined over a 16-year period (average duration of employment was 4.7–5.7 years)	TWA TDI level across jobs and times was 0.0042 ppm	No association between FVC and FEV_1 and exposure (annual or cumulative exposure measures) in smokers or nonsmokers

Table 3-3. Summary of Occupational Exposure Studies Examining the Effects of TDI on Lung Function

Study	Exposure	Effect
Peters et al. 1970 Longitudinal study 28 workers at a polyurethane foam manufacturing facility examined every 6 months for 2 years	Maximum TDI levels at examinations 1, 2, 3, and 4 were 0.003, 0.012, 0.0015, and 0.0145 ppm, respectively	FEV_1 levels on Monday morning were significantly lower at the second examination than first examination
Peters et al. 1968 Cross-sectional study 38 workers at a polyurethane foam manufacturing facility	TDI levels ranged from 0.0001 to 0.003 ppm (levels were approximately 10-fold higher the previous year)	Decreased FVC, FEV_1, $FR_{50\%}$, and $FR_{25\%}$ during the workday
Świerczyńska-Machura et al. 2015 Cross sectional study 30 workers at polyurethane foam manufacturing facility	Arithmetic mean TDI levels at different work areas ranged from 0.0005 to 0.0037 ppm	Changes indicative of mild bronchial obstruction noted in 17% of workers
Wegman et al. 1977 Longitudinal study 57 polyurethane foam manufacturing workers originally examined in 1972 were re-examined in 1974	TDI levels were ≤0.0015, 0.0025-0.0030, and ≥0.0035 ppm	Decline in FEV_1 was dose-related and exceeded predicted levels in the highest two groups
Wegman et al. 1982 Longitudinal study 48 TDI workers from Wegman et al. (1977) re-examined in 1976	TDI levels were ≤0.0015, 0.0025-0.0030, and ≥0.0035 ppm	4-Year change in FEV_1 was significantly greater in ≥0.0035 ppm group than low exposure group; most of the decline occurred in the first 2 years of the study
White et al. 1980 Cross-sectional study 147 machinists making seat covers with a fabric backed with flame-bonded polyurethane foam; 30% workers reported wheezing and/or dyspnea	TDI levels ranged from 0.0003 to 0.003 ppm	Higher prevalence of peak flow rates <90% of predicted, as compared to 45 workers who never machined polyurethane foam

$FEF_{25–75\%}$ = forced expiratory flow between 25 and 75% of FVC; FEV_1 = forced expiratory volume in 1 second; FEV_1% = ratio of FEV_1 to FVC; $FR_{50\%}$ = flow rate at 50% vital capacity; $FR_{25\%}$ = flow rate at 25% vital capacity; FVC = forced vital capacity; MMF = maximal mid-expiratory flow; PEF = peak expiratory flow; TDI = toluene diisocyanate; TWA = time-weighted average

increase in annual declines in FEV_1 and FVC among workers at a polyurethane foam manufacturing facility who entered the study with no prior exposure to TDI (naïve group). It was noted that the greatest decline in lung function occurred during the first few months of employment. The mean 8-hour TWA TDI level for the entire cohort of exposed workers was 0.0012 ppm. No exposure-related effects on lung function were found in the whole cohort of exposed workers. A follow-up of this cohort (Clark et al. 2003) did not find significant increases in annual declines in lung function in the whole cohort or the naïve group in subsequent years. Another study of polyurethane manufacturing workers found larger-than-expected annual losses of MMF, ratio of FEV_1 to FVC (FEV_1%), and PEF in workers with an 8-hour TWA TDI level of 0.0082 ppm with maximal TWA peak concentrations of 0.02–0.03 ppm (Omae et al. 1992). Annual declines in lung function parameters were not observed in workers with an 8-hour TWA TDI level of 0.0017 ppm with maximal TWA peak concentrations of 0.0003–0.004 ppm. In another study of a new polyurethane foam production facility, no significant alterations in FEV_1, FVC, or FEV_1/FVC ratio were found after 6 or 12 months of exposure (Gui et al. 2014). However, it was noted that in 9.1% of the 33 workers, there was a decrease in FEV_1 of >15% between 6 and 12 months of exposure. TWA TDI levels were not reported; the investigators noted that the TDI levels in >90% of the air samples were below the detection limit of 0.0001 ppm.

Other longitudinal studies of workers at TDI manufacturing facilities (Adams 1975; Bodner et al. 2001; Butcher et al. 1977; Ott et al. 2000) or polyurethane foam manufacturing facilities (Clark et al. 2003; Jones et al. 1992) have not found significant associations between TDI exposure and declines in lung function (Table 3-3). In longitudinal analysis of FVC and FEV_1 levels and TDI exposure, Ott et al. (2000) did not find statistically significant associations in TDI manufacturing workers with a TWA TDI level of 0.0042 ppm. Similar findings were reported in another study of TDI manufacturing workers with a mean TDI exposure level of 0.0023 ppm (Bodner et al. 2001). Ott et al. (2003) concluded that among nonsensitized TDI workers, exposure to ≤5 ppb (8-hour TWA) was not associated with decreases in FEV_1. However, it is noted that the investigators did not consider the possibility of increased toxicity among naïve workers, as found by Clark et al. (1998) and Diem et al. (1982).

Cross-sectional studies, summarized in Table 3-3, also examined declines in lung function due to TDI exposure (Huang et al. 1991a, 1991b; Olsen et al. 1989; Peters et al. 1968; Świerczyńska-Machura et al. 2015; White et al. 1989). The interpretation of the results of some of the studies is limited by the inclusion of workers with asthma-like respiratory symptoms (Huang et al. 1991a, 1991b; White et al. 1980) or the lack of controls (Świerczyńska-Machura et al. 2015). Olsen et al. (1989) did not find associations between TDI exposure (current exposure, highest career exposure, cumulative exposure, or

cumulative highest-to-date exposure) and FEV_1 levels in a study of TDI manufacturing workers. Although the TDI levels were not reported, the investigators noted that TDI levels at the facility did not exceed the permissible level of 0.02 ppm; the investigators did not provide definitions of highest career exposure, cumulative exposure, or cumulative highest-to-date exposure or information on how they were calculated. In a study of polyurethane foam manufacturing workers, Peters et al. (1968) reported significant decreases in FVC, FEV_1, flow rate at 50% vital capacity ($FR_{50\%}$), and $FR_{25\%}$ when end-of-shift values were compared to start-of-shift values. Further declines in FVC, peak flow rate (PFR), $FR_{50\%}$, and $FR_{25\%}$ were found when Monday afternoon values were compared to Monday morning values. The TDI levels ranged from 0.0001 to 0.003 ppm.

Animal studies support the findings from human studies that the respiratory tract is a sensitive target of TDI toxicity. Signs of irritation and histological damage have been observed following acute-, intermediate-, and chronic-duration exposure. Acute studies in mice and rats demonstrated that TDI was a sensory irritant and that the level of response was related to the concentration and the duration of exposure (Pauluhn 2014; Sangha and Alarie 1979). RD_{50} values (concentration resulting in a 50% decrease in respiration rate) in mice exposed for 10, 30, 60, 120, 180, or 240 minutes were 0.813, 0.498, 0.386, 0.249, 0.199, and 0.199 ppm, respectively (Sangha and Alarie 1979). When the animals were repeatedly exposed, the RD_{50} values were lower on subsequent days; after 3 days of exposure to 0.023–1.18 ppm, pre-exposure respiratory rates were lower than day 1, indicating an incomplete recovery.

Histological alterations have been observed in the nasal cavity (Arts et al. 2008; Buckley et al. 1984; Johnson et al. 2007; Loeser 1983; Matheson et al. 2005; Sangha and Alarie 1979; Zissu 1995), trachea (Gordon et al. 1985), and lung (Loeser 1983; Wong et al. 1985) in laboratory animals. The nasal lesions consist of inflammation, hyperplasia, degeneration, ulceration, and metaplasia; the severity and the location of the damage appear to be concentration and duration related. At lower concentrations and shorter durations, only the nares (most anterior region of the nasal cavity) are affected; at high concentrations, the damage extends to the olfactory epithelium. The only NOAEL identified for nasal effects is 0.031 ppm in mice exposed 3 hours/day for 5 days (Sangha and Alarie 1979). When the duration was extended to at least 6 weeks (4 hours/day, 5 days/week), exudate, goblet cell metaplasia, and inflammation were observed in the nares of mice exposed to 0.02 ppm (Matheson et al. 2005). Rhinitis was also observed in rats exposed to 0.02 ppm in a 2-generation study (Tyl et al. 1999b). Exposure to 0.05 ppm 4 hours/day for 12 days resulted in extensive inflammatory cell infiltration into the lamina propria in the nasoturbinates and maxilloturbinates of mice; goblet cell metaplasia was also observed in the maxilloturbinates (Johnson et al. 2007). Extending exposure to 0.07 ppm from 4 to 9 days resulted in

an increase in the severity of the rhinitis and respiratory epithelia metaplasia and necrosis observed in mice (Zissu 1995). The TDI concentration associated with olfactory epithelial damage was 0.4 ppm (6 hours/day for 5 days), which resulted in moderate olfactory epithelial ulceration and necrosis and slight degeneration of the olfactory nerve (Buckley et al. 1984). Concentration-related increases in the incidence and severity of chronic or necrotic rhinitis with epithelial atrophy and mucous and squamous metaplasia were observed in mice exposed to ≥0.05 ppm TDI for 2 years (Loeser 1983).

Slight laryngeal epithelial hyperplasia was observed in mice exposed to 1.0 ppm TDI for 6 hours (Arts et al. 2008). Exposure of guinea pigs to 2 ppm for 1 hour resulted in patchy loss of cilia and disruption of the surface epithelium in the trachea (Gordon et al. 1985). Matheson et al. (2005) reported goblet cell inflammation, epithelial hyperplasia, regeneration, and loss of structure in the lungs of mice exposed to 0.5 ppm for 2 hours. However, other acute studies at similar (0.4 ppm 6 hours/day for 5 days) or higher (1 ppm for 6 hours) concentrations did not find histological alterations in the lungs (Arts et al. 2008; Buckley et al. 1984). Interstitial inflammation, localized pleural thickening, and goblet cell hyperplasia were observed in the lungs of guinea pigs 50 days after exposure to 1.4 ppm 3 hours/day for 5 days (Wong et al. 1985). Similarly, goblet cell metaplasia in the central airway mucosa and eosinophil infiltration in the central and peripheral airways were observed in rats exposed to 0.41 ppm 2,4-TDI 4 hours/day for 5 days (Kouadio et al. 2014); the severity of the lesions increased with exposure concentration. In a chronic mouse study, interstitial pneumonitis and catarrhal bronchitis were observed in "some mice," with a higher incidence at 0.15 ppm (Loeser 1983).

In addition to the histological alterations, studies in guinea pigs, rats, and mice have demonstrated TDI sensitization (Aoyama et al. 1994), bronchial hyperresponsiveness (Gagnaire et al. 1996; Gordon et al. 1985; Kouadio et al. 2014; Marek et al. 1999; Matheson et al. 2005), and nasal hyperresponsiveness (Kouadio et al. 2014). An increase in respiratory rate was observed in guinea pigs exposed to 0.2 ppm TDI 3 hours/day for 5 days and challenged with 0.02 ppm TDI (15-minute exposure) 26 days after the initial exposure (Aoyama et al. 1994). The TDI challenge concentration did not elucidate a response in guinea pigs previously exposed to 0.02 ppm for 5 days. Challenge tests with acetylcholine or methacholine resulted in airway hyperresponsiveness in guinea pigs and mice previously exposed to a TDI concentration as low as 0.01 ppm (6 hours/day for 5 days) (Marek et al. 1999); a NOAEL of 0.005 ppm was identified in the same study. Airway hyperresponsiveness was also observed following a 1-hour exposure to a relatively high concentration (3 ppm) (Gagnaire et al. 1996). The effects were observed 30 minutes after exposure and persisted for 48 hours. A TDI challenge following 4-hour/day exposure to 2,4-TDI resulted in severe labored breathing as evidenced by gasping and breathing with an

open mouth after 4 days of exposure to 1.14 ppm (Kouadio et al. 2014); the severity of the labored breathing was less severe after 2 or 3 exposure days. Labored breathing was also observed in rats similarly exposed to 0.41 ppm for 4 or 5 days. Symptoms of nasal hyperresponsiveness (sneezing and hyperrhinorrhea) were also observed at both concentrations (Kouadio et al. 2014).

MDI. The toxicity of MDI to the respiratory tract has not been as well investigated as TDI, but the effects appear to be similar. The primary effects observed include occupational asthma in sensitized individuals and decreased lung function. MDI-induced asthma has been reported in occupational exposure studies and case reports (Bonauto et al. 2005; Burge 1982; Chang and Karol 1984; Helaskoski et al. 2015; Hur et al. 2008; Liss et al. 1988; Suojalehto et al. 2011; Woellner et al. 1997; Zammit-Tabona et al. 1983); however, no reliable dose-response data are available. Asthma symptoms were noted in 18 of the 106 workers at a wood products plant using heated MDI in the manufacture of synthetic wood (Woellner et al. 1997). Symptoms occurred within the first 12 months of operation; half reported symptoms within the first 7 months of operation when operational problems would likely have resulted in MDI levels that exceeded the permissible level of 0.02 ppm. Bonauto et al. (2005) used worker's compensation claims to estimate the rate of asthma in the spray-on truck bed lining industry. A rate of 200 per 10,000 full-time employees was found; however, no testing was done on any of the claims to determine whether MDI was causative agent. Approximately half of subjects reporting asthma-like symptoms (wheezing, dyspnea, and/or cough) had a positive response in a methacholine-challenge test. A study of 11 foundry workers with asthma-like symptoms found that 6 had a positive response in a MDI-challenge test (Zammit-Tabona et al. 1983). Another study of 40 MDI workers found that 24 responded to MDI challenge at test atmospheres of up to 0.02 ppm (Burge 1982). A third study (Hur et al. 2008) diagnosed MDI-induced asthma or eosinophilic bronchitis in 6 of 13 car upholstery factory workers with lower respiratory symptoms. The incidence of nonspecific bronchial hyperresponsiveness to a challenge with methacholine was significantly higher among MDI workers with asthma-like symptoms, as compared to controls and TDI workers (Jang et al. 2000). Several case reports of MDI workers (Bascom et al. 1985; Baur et al. 1984; Malo and Zeiss 1982; Zeiss et al. 1980) and a study of MDI workers (Baur 1995) with asthma-like symptoms also reported chills, fever, and malaise, which are consistent with symptoms of hypersensitivity pneumonitis.

Several occupational exposure studies have examined the effect of MDI exposure on lung function (Liss et al. 1988; Musk et al. 1982; Sulotto et al. 1990). Lung function was examined in 27 polyurethane foam workers who were asymptomatic for asthma; the MDI concentrations ranged from 0.0005 to 0.001 ppm. A comparison of lung function values of the workers to an age-matched control group of 27 clerks did not

show statistically significant differences between the groups. Additionally, no differences in the change in lung function over a work week (Monday values compared to Friday values) or over the work shift were found. Musk et al. (1982) examined 107 workers at two polyurethane plastic manufacturing facilities over a 5-year period; 25 of the subjects were only exposed to MDI, 17 to only TDI, 6 to MDI and TDI, and 42 were not exposed to diisocyanates. The geometric mean MDI levels reported by the plants during the last year of the study were 0.0006 and 0.0003 ppm at plants 1 and 2, respectively; however, the mean MDI levels measured by the investigators ranged from 0.001 to 0.003 ppm. It should be noted that MDI exists as an aerosol; thus, the method used to measure MDI air levels (impinged method) may have underestimated MDI levels (EPA 1998a). Lung function tests were conducted on Monday morning and afternoon and on Monday morning and afternoon following a 2-week vacation. No significant alterations in FEV_1 or FVC levels were found over the 5-year period, over a workday, or after a 2-week non-exposure period among the MDI-only exposed group.

Several studies of MDI workers have examined the possible association between specific immunoglobulin antibodies to MDI and MDI-induced asthma (Hur et al. 2008; Pezzini et al. 1984; Tse et al. 1985; Zeiss et al. 1980). In a study of 76 foundry workers (10 with asthma-like symptoms and bronchial hyperreactivity, 40 with respiratory symptoms but no evidence of bronchial hyperreactivity, and 26 with no respiratory symptoms), specific IgE antibodies to MDI-HSA conjugates were found in 2 workers (1 with asthma-like symptoms and one with other symptoms) and specific IgG antibodies to MDI-HSA conjugates were found in 5 workers (3 with asthma-like symptoms, 1 with other symptoms, and 1 with no symptoms) (Tse et al. 1985). Another study of car upholstery factory workers found specific IgG antibodies to MDI-HSA conjugates in 20.7% of the 58 workers (4/12 of the responders were diagnosed with MDI-induced asthma or eosinophilic bronchitis) and specific IgE antibodies to MDI-HSA conjugates in 8.6% of the workers (2/5 of the responders were diagnosed with MDI-induced asthma or eosinophilic bronchitis) (Hur et al. 2008).

A limited number of laboratory animal studies have examined the toxicity of MDI to the respiratory tract. An RD_{50} of 32 mg/m^3 in mice exposed to 4,4'-MDI for 4 hours was calculated (Weyel and Schaffer 1985). Exposure to concentrations as low as 7 mg/m^3 initially resulted in increases in respiratory rate followed by a gradual decline in respiratory rate; a similar respiratory pattern was observed in mice administered 4,4'-MDI via tracheal cannulation. The investigators suggested that this respiratory pattern was indicative of a pulmonary irritant rather than a sensory irritant. Increases in airway hyper-responsiveness to acetylcholine were observed in guinea pigs exposed to 0.01 ppm MDI 6 hours/day for

5 days or 6 hours/day, 5 days/week for 4 weeks (Marek et al. 1999); a NOAEL of 0.005 ppm was identified in the 5-day study.

In a chronic-duration study (Reuzel et al. 1994), rats were exposed to polymeric MDI (containing 44.8–50.2% monomeric MDI) for 1 or 2 years. After 2 years of exposure, nasal and pulmonary lesions were observed at 1.0 and 6.0 mg/m^3 and no alterations were observed at 0.2 mg/m^3. The nasal lesions consisted of basal cell hyperplasia and Bowman's gland hyperplasia in males at ≥1.0 mg/m^3 (basal cell hyperplasia was also observed in females at 6.0 mg/m^3) and minimal to severe olfactory epithelial degeneration in males and females at 6.0 mg/m^3; after 1 year of exposure, the only nasal lesion with a significantly increased incidence was minimal to moderate olfactory epithelial disarrangement in males exposed to 6.0 mg/m^3. The lung lesions in rats exposed to 1.0 or 6.0 mg/m^3 for 2 years consisted of mild to moderate localized fibrosis and alveolar duct epithelialization; exposure to 6.0 mg/m^3 also resulted in localized alveolar bronchiolization. Additionally, an accumulation of macrophages with yellow pigment was observed at 1.0 and 6.0 mg/m^3. After 1 year of exposure, minimal to moderate localized fibrosis, alveolar duct epithelialization, and localized alveolar bronchiolization were observed at 6.0 mg/m^3; alveolar duct epithelialization was also observed in the females exposed to 1.0 mg/m^3.

An unpublished study conducted by Hoyemann and associates in 1995 also evaluated the chronic toxicity of MDI in rats; this study was reviewed by Feron et al. (2001). Groups of 80 female Wistar rats were exposed to 0, 0.23, 0.70, or 2.05 mg/m^3 monomeric MDI aerosols (mass median aerodynamic diameter [MMAD] of approximately 1.0 µm) 18 hours/day, 5 days/week for approximately 2 years. As reviewed by Feron et al. (2001), significant increases in absolute and relative lung weight were observed at 2.05 mg/m^3. A number of histological alterations were observed at 0.23 mg/m^3 including bronchiolo-alveolar hyperplasia, mononuclear cell infiltration, and fibrosis; the incidence and severity of the lesions appeared to be concentration related. These effects are similar to those observed in Reuzel et al. (1994). The LOAELs from the two studies are similar after adjusting for intermittent exposure: 0.178 mg/m^3 for the Reuzel et al. (1994) study and 0.123 mg/m^3 for the Hoyemann study.

Cardiovascular Effects.

TDI. No histological alterations were observed in the aorta or heart of rats or mice exposed to 0.15 ppm commercial-grade TDI for 2 years (Loeser 1983).

MDI. No histological alterations were observed in the heart of rats exposed to 6.0 mg/m^3 polymeric MDI for 2 years (Reuzel et al. 1994).

Gastrointestinal Effects.

TDI. Workers with accidental exposure to unknown quantities of TDI spilled from tanks have reported nausea and vomiting during exposure (Axford et al. 1976; Singer and Scott 1987).

Shadnia et al. (2013) reported a case of intestinal obstruction in a 16-year-old male worker in an Iranian sponge production factory. The subject's symptoms began after he was exposed to TDI for 2 hours; details of his exposure prior to this incident, or coexposures during the incident, were not provided. The authors noted that the subject had a past history of surgery for stomach lymphoma, and during surgery to correct the obstruction, mild adhesions from the previous surgery were seen. However, the authors noted that the surgery did not identify any possible causes of the obstruction; they postulated several possible mechanisms by which TDI may have induced the effect, including triggering an inflammatory response, interrupting parasympathetic nervous system function, or decreasing bowel motility via an effect on intestinal smooth muscle.

No gastrointestinal lesions were observed in rats or mice exposed to 0.15 ppm commercial-grade TDI for 2 years (Loeser 1983).

MDI. No histological alterations were observed in the gastrointestinal tract of rats exposed to 6.0 mg/m^3 polymeric MDI for 2 years (Reuzel et al. 1994).

Hematological Effects.

TDI. No hematological alterations were noted in rats or mice exposed to 0.15 ppm commercial-grade TDI for 2 years (Loeser 1983).

MDI. No hematological alterations were observed in rats exposed to 6.0 mg/m^3 polymeric MDI for 2 years (Reuzel et al. 1994).

Musculoskeletal Effects.

TDI. No histological alterations were observed in the femur or skeletal muscle (quadriceps) of rats or mice exposed to 0.15 ppm commercial-grade TDI for 2 years (Loeser 1983).

MDI. Reuzel et al. (1994) examined 43 organs and tissues, which likely included bones and skeletal muscles, of rats exposed to 6.0 mg/m^3 polymeric MDI for 2 years and did not find histological alterations outside of the respiratory tract.

Hepatic Effects.

TDI. No histological alterations in the liver or alterations in serum chemistry parameters were observed in rats or mice exposed to 0.15 ppm commercial-grade TDI for 2 years (Loeser 1983).

MDI. No histological alterations were observed in the liver of rats exposed to 6.0 mg/m^3 polymeric MDI for 2 years (Reuzel et al. 1994); additionally, no alterations in serum clinical chemistry parameters were observed.

Renal Effects.

TDI. No histological alterations were observed in the kidneys in rats or mice exposed to 0.15 ppm commercial-grade TDI for 2 years (Loeser 1983).

MDI. No histological alterations in the kidneys or alterations in urinalysis parameters were observed in rats exposed to 6.0 mg/m^3 polymeric MDI for 2 years (Reuzel et al. 1994).

Dermal Effects.

TDI. In a study of 114 workers at a flexible foam manufacturing facility (Daftarian et al. 2002), production workers who were exposed to TDI reported skin conditions such as dermatitis, eczema, or other red rash in the previous 12 months more than twice as often as unexposed non-production workers (prevalence rate ratio of 2.66; 95% confidence interval [CI] 1.14–16.32, $p<0.02$). Skin patch testing and blood samples for specific IgG or IgE antibodies to TDI were largely negative (only 2/100 workers had a

positive result to any of these tests, and only for specific IgG); thus, the effects were considered to be related to irritation rather than an immune response.

MDI. Stingeni et al. (2008) reported a case of facial urticaria in a worker using a polyurethane glue containing MDI.

Ocular Effects.

TDI. In a case series of ocular effects in humans or animals exposed to diisocyanates, Luckenbach and Kielar (1980) evaluated visual acuity and ophthalmologic status in nine workers exposed to TDI during the production of polyurethane foam. No information on exposure levels was provided; the various cases had worked in the facility for durations ranging from 10 days to 2 years. All nine workers reported ocular symptoms such as “smoky” or “foggy” vision or eye irritation, usually resolving during weekends or overnight. In all nine, microcystic edema of the corneas and conjunctival injection (dilation of conjunctival blood vessels leading to appearance of redness) were observed upon ophthalmologic examination, with more severe cases associated with diminished visual acuity. None of the cases exhibited abnormal Schirmer I tear test or tear break-up time (Luckenbach and Kielar 1980).

Littorin et al. (2007) noted a significant association between self-reported eye symptoms and continuous measures of TDI exposure in workers. When the 2,4-TDI and 2,6-TDI levels were examined separately, a stronger association was found between eye symptoms and 2,4-TDI levels than with 2,6-TDI levels.

MDI. No information was located regarding ocular effects in humans or animals following inhalation exposure to MDI.

Body Weight Effects.

TDI. Significant decreases (45% relative to controls) in maternal body weight gain were seen in CD rats during exposure (6 hours/day on GDs 6–15) to 0.5 ppm commercial-grade TDI in a developmental toxicity study (Tyl et al. 1999a); in a range-finding study, significant weight loss was observed at 1 ppm. In a 2-generation reproductive toxicity study, intermediate-duration exposure (19 weeks including premating, mating, gestation, and lactation) to 0.3 ppm commercial-grade TDI did not alter body weight of male or female F0 or F1 parental CD rats (Tyl et al. 1999b). Chronic exposure of rats to 0.15 ppm commercial-grade TDI resulted in significant reductions in body weight gain (Loeser et al. 1983).

However, the magnitude of change was not reported; the investigators did not report any changes in body weight gain at 0.05 ppm.

MDI. Acute-duration exposure of Wistar rats to 4,4'-MDI during gestation (6 hours/day on GDs 6–15) did not alter maternal body weight or body weight gain at exposure concentrations up to 9 mg/m^3 (Buschmann et al. 1996). No significant alterations in body weight gain were observed in rats exposed to 6.0 mg/m^3 polymeric MDI for 2 years (Reuzel et al. 1994).

3.2.1.3 Immunological and Lymphoreticular Effects

Although the exact mechanism of toxicity of TDI and MDI has not been elucidated, there is some indication that occupational asthma observed in some workers has an immune component, and several studies have reported alterations in TDI or MDI specific IgG and IgE antibodies in workers with asthma (Baur and Fruhmann 1981; Cvitanovic et al. 1989; Hur et al. 2008; Park and Nahm 1996; Park et al. 1999; Pezzini et al. 1984; Sharifi et al. 2013; Tse et al. 1985; Zeiss et al. 1980). These findings are discussed in detail in the Respiratory Effects section.

3.2.1.4 Neurological Effects

TDI. Le Quesne et al. (1976) described the immediate and long-term neurological effects in 23 firefighters who were "heavily exposed" during a fire at a polyurethane foam factory; approximately 4,500 L of TDI had spilled from storage tanks during the fire (Axford et al. 1976). The firefighters were exposed via inhalation, and some also had dermal contact. Other chemicals were stored at the factory, but the spillage was apparently limited to TDI; however, exposure to other chemicals cannot be ruled out. Additionally, exposure to carbon monoxide or anoxia may have contributed to the observed effects; the investigators noted that there was no evidence that the fumes were sufficiently dense for the firefighters to become anoxic. Five of the exposed men reported symptoms during the fire including euphoria, ataxia, and transient loss of consciousness; two reported headache the next day. Seventeen of the firefighters were medically evaluated 3 weeks later and 14 of these men reported symptoms of confusion, poor memory, headache, irritability, difficulty concentrating, or depression. Neurological examination showed slight changes including ataxia and mild sensory loss; electroencephalography recordings on nine men were unremarkable. At follow-up of 18 men 4 years later, memory problems were still reported by most of the subjects, and some reported persistence of concentration difficulty, irritability, and depression. No abnormalities were seen on neurological examination; however, a statistically significant ($p<0.02$) impairment in long-term recall was noted in the Wechsler memory scale when tests in exposed men who

reported persistent effects were compared with unexposed firemen from another area (Le Quesne et al. 1976).

Singer and Scott (1987) reported neurological symptoms and neuropsychological and electrophysiology test results for three male wharf workers who were exposed to spilled TDI. Both dermal contact and inhalation exposure were described, and the total duration of exposure was about 4 hours for all three workers. The workers reported feeling dizzy during exposure. The workers were evaluated 2 and 16 months after exposure using neuropsychological tests and nerve conduction velocity measurements. Test results showed statistically significant decreases ($p<0.0003$) in verbal, performance, and full-scale IQ at 16 months postexposure compared with 2 months postexposure (full scale IQ dropped between 20 and 26 points in all three subjects); while the tests administered at each time were slightly different (WAIS at 2 months versus WAIS-R at 16 months), on average, these tests differ only by 7–8 points (Singer and Scott 1987). In addition to IQ change, statistically significant impairments in both the Benton Visual Retention and Wechsler Memory Scale: Logical Memory were observed. Two of the three subjects exhibited significantly reduced nerve conduction velocities, one in the median sensory nerve and the other in the sural nerve, while the third showed no change in nerve conduction. The study authors also reported that testing at 16 months postexposure showed severe deficits in manual dexterity, visuomotor tracking, mental flexibility, ability to detect figure-ground relationships, and word fluency (additional details of these findings were not provided). The small number of subjects, lack of a control group, and small magnitude of the effects limit the interpretation of the results.

Hughes et al. (2014) recently evaluated the available data on the neurotoxicity of diisocyanates to determine whether a causal association could be established between diisocyanate exposure (the studies involved exposure to TDI, MDI, HDI, or unspecified diisocyanates) and neurotoxicity. Using the Hill criteria for causality, Hughes et al. (2014) concluded that there was limited evidence for strength of association and consistency, and the data were inadequate to establish a casual association between diisocyanates and neurotoxicity. The investigators noted several limitations of the studies included in their systematic review such as limited exposure information (including the lack of objective exposure measures and no dose-response assessment), co-exposure to known neurotoxicants, and lack of objective measures of neurotoxicity. Additionally, they noted that no plausible mechanisms of toxicity were identified.

No animal studies have examined the potential of TDI to induce neurological effects. No histological alterations were observed in the brain, sciatic nerve, or spinal cord of rats or mice exposed to 0.15 ppm commercial-grade TDI for 2 years (Loeser 1983).

MDI. Information on the neurotoxicity of MDI is limited to a chronic study that did not find histological alterations in the brain of rats exposed to 6.0 mg/m^3 polymeric MDI for 2 years (Reuzel et al. 1994).

3.2.1.5 Reproductive Effects

TDI. No information was located regarding reproductive effects in humans following inhalation exposure to TDI. When groups of 28 CD rats were exposed via inhalation to commercial-grade TDI (80:20 mix of 2,4- and 2,6-TDI), no effects on reproductive toxicity parameters (including mating, fecundity, or fertility indices; gestation length; numbers of live litters or live birth indices; gross necropsy findings; or histopathology of reproductive organs) were seen at exposures up to 0.3 ppm, 6 hours/day, 5 days/week for 2 generations (Tyl et al. 1999b). A 2-year study in rats and mice did not find histological alterations in the gonads of male and female rats and mice (Loeser 1983).

MDI. No information was located regarding reproductive effects in humans following inhalation exposure to MDI. A 2-year study in male and female rats did not find histological alterations in the gonads at 6.0 mg/m^3 polymeric MDI (Reuzel et al. 1994).

3.2.1.6 Developmental Effects

TDI. No information was located regarding developmental effects in humans following inhalation exposure to TDI. Exposure of rats to 0.5 ppm TDI (80:20 mix of 2,4- and 2,6-TDI) during GDs 6–15 resulted in an increased incidence of litters with poorly ossified cervical centrum no. 5, but no other treatment-related increases in anomalies or variations (Tyl et al. 1999a). Maternal toxicity, including markedly reduced body weight gain and respiratory symptoms, was seen at 0.5 ppm, and the observed developmental effects may have been secondary to the maternal toxicity.

MDI. No information was located regarding developmental effects in humans following inhalation exposure to MDI. After exposure of rats to 9 mg/m^3 4,4'-MDI aerosol for 6 hours/day on GDs 6–15, there was an increase in the incidence of litters with fetuses displaying asymmetric sternebrae (10/23 litters versus 2/25 control litters, $p<0.05$); no effects of treatment were seen on other gestational parameters or on malformation or variation incidences at lower exposure concentrations (Buschmann et

al. 1996). The investigators noted that the incidence was within the limits of biological variability. Apart from reduced food consumption, dams did not exhibit signs of toxicity at any exposure concentration in this study; an increase in lung weight was also observed in the dams.

3.2.1.7 Cancer

Human data on the potential association between inhalation exposure to diisocyanates and cancer are available from studies of three cohorts of workers engaged in polyurethane foam manufacture. Studies of these cohorts (Mikoczy et al. 2004; Schnorr et al. 1996; Sorahan and Nichols 2002) have suggested an association between work in the polyurethane foam manufacturing industry and lung cancer in female workers, but an association with diisocyanate exposure was not established. Significant limitations of all three studies included lack of control for confounding factors such as smoking and alcohol consumption and coexposure to mixtures of compounds including those other than diisocyanates.

Cohort studies of cancer and diisocyanate exposure include: a cohort of 4,611 workers from 4 plants in the United States (Schnorr et al. 1996); a cohort of 4,175 workers from 9 plants in Sweden (Mikoczy et al. 2004; Hagmar et al. 1993a, 1993b); and a cohort of 8,288 workers in 11 plants in the United Kingdom (Sorahan and Nichols 2002; Sorahan and Pope 1993). Sorahan and Nichols (2002) reported data on the largest number of person-years at risk (200,262); Schnorr et al. (1996) reported on 90,393 person-years at risk, and Mikoczy et al. (2004) reported on 83,023 person-years at risk. None of the studies provided quantitative estimates of individual exposures. Workers in all three cohorts were exposed to a mixture of TDI isomers, and those at some plants were also exposed to unspecified isomers of MDI. In addition, all of the cohorts included workers who may have been exposed to other airborne contaminants such as methylene chloride, aliphatic amines, acrolein, acrylonitrile, styrene, amine accelerators such as bis(2-dimethylaminoethyl) ether, and others (Mikoczy et al. 2004; Schnorr et al. 1996).

Table 3-4 shows the results of the most recent studies in the three cohorts. Increased standardized mortality ratios (SMRs) for lung cancer were reported for women in all three cohorts; the increases were statistically significant in the U.K. (Sorahan and Nichols 2002) and Swedish (Mikoczy et al. 2004) cohorts, but not the U.S. cohort (Schnorr et al. 1996). Mikoczy et al. (2004) also reported a significantly increased incidence of lung cancer in females (standardized incidence ratio [SIR] of 3.0; 95% CI 1.55–5.24) compared with the expected incidence in the general Swedish population. However, when stratified

Table 3-4. Results of Cohort Studies of Diisocyanate Exposure and Mortality from Specific Cancers

	U.S. cohort		U.K. cohort		Swedish cohort	
Reference	Schnorr et al. 1996		Sorahan and Nichols 2002		Mikoczy et al. 2004	
Cohort size (number of plants)	4,611 (4)		8,288 (11)		4,175 (9)	
Time period of follow-up	1958–1993		1958–1998		1959–1998	
Person-years at risk	90,393		200,262		83,023	
Cancer site	Number of cases	SMR (95% CI)	Number of cases	SMR (95% CI)	Number of cases	SMR (95% CI)
Females						
Lung	8	173	35	181[a] (126–251)	10	352[a] (169–648)
Rectum	0	NA	2	53 (6–192)	–	–
Non-Hodgkin's lymphoma	–	–	3	110 (23, 321)	–	–
Hodgkin's disease	–	–	0	NA	–	–
Males						
Lung	12	79	134	107 (90–127)	7	49 (20–101)
Rectum	3	390	10	65 (31–120)	–	–
Non-Hodgkin's lymphoma	–	–	6	65 (24–142)	–	–
Hodgkin's disease	–	–	1	44 (1–243)	–	–
Females and males (combined)						
Lung	20[b]	101 (62–156)	–	–	17	99 (58–159)
Rectum	3	278 (57–813)	–	–	–	–
Non-Hodgkin's lymphoma	4	154 (42–395)	–	–	–	–
Hodgkin's disease	2	232 (28–838)	–	–	–	–

[a]Significantly different from null hypothesis at $p<0.05$.
[b]Includes tumors of the lung, trachea, and bronchus.

– = not reported; CI = confidence interval; SMR = standardized mortality ratio

by “apparent” versus “no or low” exposure to TDI or MDI, women with “apparent” exposure did not exhibit a higher risk of lung cancer. In addition, Mikoczy et al. (2004) conducted a nested case-referent study of the 12 lung cancer cases among female workers, and observed no greater prevalence of exposure to polyurethane dust in cases compared with referents. Similarly, Sorahan and Nichols (2002) observed a statistically significant increased incidence of lung and bronchus cancer in women (standardized registration ratios [SRR] 199; 95% CI 135–282), but reported that all of the female lung cancers in the cohort were in women who did not work for any period in an isocyanate-exposed setting. Schnorr et al. (1996) did not evaluate the effect of isocyanate exposure duration on lung cancer risk in women alone, but in both male and female workers, there was no trend of increased lung cancer mortality by duration of exposure or time since first exposure.

Schnorr et al. (1996) reported nonsignificant increases in the SMRs for rectal cancer, Hodgkin’s disease, and non-Hodgkin’s lymphoma in the U.S. cohort; however, studies of the Swedish and U.K. cohorts (Mikoczy et al. 2004 and Sorahan and Nichols 2002, respectively) did not support these findings, as SMRs for these neoplasms were reduced in the exposed workers of these cohorts (Table 3-4).

TDI. When groups of 126/sex Sprague-Dawley rats and 120/sex CD-1 mice were exposed to vapors of commercial-grade TDI via whole-body inhalation on 6 hours/day, 5 days/week for 108–110 weeks (Loeser 1983), there were no treatment-related increased tumor incidences in rats or mice.

The authors noted that histopathology of the nasal turbinates in rats was still in progress, but that there were no grossly visible effects of treatment on the upper respiratory tract. No follow-up study was identified in the literature search. This study lacked some details in methodology, and did not describe the approach to statistical analysis.

MDI. Groups of 60 Wistar rats per sex were exposed to aerosolized polymeric MDI at nominal concentrations of 0, 0.2, 1.0, or 6.0 mg/m^3 via whole-body inhalation 6 hours/day, 5 days/week for 2 years (Reuzel et al. 1994). A significantly increased incidence (6/60) of lung adenoma, as well as one lung adenocarcinoma, was observed in male rats exposed to 6.0 mg/m^3 polymeric MDI. No lung tumors occurred in control, 0.2, or 1.0 mg/m^3 exposure groups of male or female rats. In female rats exposed to 6.0 mg/m^3, 2/59 animals developed lung adenomas; there were no adenocarcinomas (Reuzel et al. 1994).

In a second study conducted by Hoyemann and associates (reviewed by Feron et al. 2001) in which female Wistar rats were exposed to monomeric MDI 18 hours/day, 5 days/week for 2 years, the

occurrence of lung tumors was limited to a bronchiolo-alveolar adenoma observed in 1/80 rats exposed to 2.05 mg/m^3.

3.2.2 Oral Exposure

It is noted that TDI and MDI are rapidly hydrolyzed in aqueous environments and it is unlikely that humans will be exposed to these compounds in water. The only available information on the toxicity of TDI administered via the oral route comes from gavage studies in which rats and mice were administered TDI in corn oil for 14 days, 13 weeks, or 2 years (NTP 1986). There is some question regarding the applicability of the results of the gavage studies to humans due to likely differences in the metabolism of ingested TDI compared to gavage administered TDI. Direct instillation of TDI into the acidic stomach could result in the formation of 2,4-TDA, which is unlikely to occur following ingestion because TDI is likely to react with itself and macromolecules to form urea and polyurea in the neutral pH milieu of the mouth. No information was located regarding health effects in humans or animals following oral exposure to MDI.

The highest NOAEL values and all LOAEL values for TDI from each reliable study for each end point in each species and duration category are recorded in Table 3-5 and plotted in Figure 3-3.

3.2.2.1 Death

No information was located regarding death in humans following oral exposure to TDI. In acute-duration gavage studies of commercial-grade TDI administered in corn oil (NTP 1986), treatment-related deaths occurred at doses ≥240 mg/kg/day in rats and ≥500 mg/kg/day in mice exposed for up to 14 consecutive days; however, because sporadic deaths among male mice at lower doses (as low as 30 mg/kg/day), it is difficult to identify a clear and reliable effect level for death in mice. Data on effect levels for death of mice and rats exposed for intermediate durations are also uncertain as a consequence of sporadic deaths occurring at low doses; however, based on the study authors' determinations, treatment-related deaths of female rats and mice occurred at doses of 240 and 120 mg/kg/day, respectively (NTP 1986). In 2-year studies of commercial-grade TDI, doses ≥30 mg/kg/day reduced survival of male and female rats, while the high dose of 240 mg/kg/day reduced the survival of male mice (NTP 1986). Importantly, NTP (1986) reported that analysis of the test material in the chronic study showed that the TDI had reacted with the corn oil vehicle, yielding actual gavage doses 77–90% of nominal doses. It is reasonable to assume that similar reactions occurred in the acute- and intermediate-duration studies.

Table 3-5. Levels of Significant Exposure to Toluene Diisocyanate - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (F344/N)	single dose (G)				2150 M (2/5 M died)	NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
2	Rat (F344)	14 d, 1 x/d (G)				240 (1/5 males and 1/5 females died)	NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
3	Mouse (B6C3F1)	single dose (G)				4640 (4/5 M and 1/5 F died)	NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
Systemic								
4	Rat (F344)	14 d, 1 x/d (G)	Bd Wt	60 M	120 M (12% decrease in body weight)		NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
5	Mouse (B6C3F1)	14 d, 1 x/d (G)	Bd Wt	500			NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
INTERMEDIATE EXPOSURE								
Systemic								
6	Rat (Fischer- 344)	13 wk, 5 d/wk (G)	Bd Wt	60 M	120 M (10% decrease or greater in body weight)		NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
CHRONIC EXPOSURE								
Death								
7	Rat (F344/N)	105 wk, 5 d/wk (G)				30 (significantly decreased survival)	NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
8	Mouse (B6C3F1)	105 wk, 5 d/wk (G)				240 M (significantly decreased survival)	NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI

Table 3-5. Levels of Significant Exposure to Toluene Diisocyanate - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
9	Rat (F344/N)	105 wk, 5 d/wk (G)	Resp		30 M (bronchopneumonia)		NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
			Cardio	120 F				
			Gastro	120 F				
			Musc/skel	120 F				
			Hepatic	120 F				
			Renal	120 F				
			Bd Wt		30 M (12% decrease in terminal body weight)			

Table 3-5. Levels of Significant Exposure to Toluene Diisocyanate - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
10	Mouse (B6C3F1)	105 wk, 5 d/wk (G)	Resp	240 F			NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
			Cardio	240 F				
			Musc/skel	240 F				
			Hepatic	240 F				
			Renal	120 M	240 M (cytomegaly of tubular epithelium)			
			Endocr	240 F				
			Bd Wt	120 M	240 M (body weight decrement of ~10% throughout most of the study)			
Cancer								
11	Rat (F344/N)	105 wk, 5 d/wk (G)				60 CEL: subcutaneous fibromas/fibrosarcomas (M); pancreatic acinar cell adenomas (M); mammary gland fibroadenomas (F); pancreatic islet cell adenomas (F); neoplastic nodules of liver (F)	NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI
12	Mouse (B6C3F1)	105 wk, 5 d/wk (G)				120 F CEL: hemangiomas, hemangiosarcomas, and hepatocellular adenomas	NTP 1986 2,4/2,6-TDI	80: 20 mixture of 2,4- and 2,6-TDI

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

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; F = Female; (G) = gavage; Gastro = gastrointestinal; LOAEL = lowest-observed-adverse-effect level; M = male; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); x = time(s)

Figure 3-3. Levels of Significant Exposure to Toluene Diisocyanate - Oral

Acute (≤14 days)

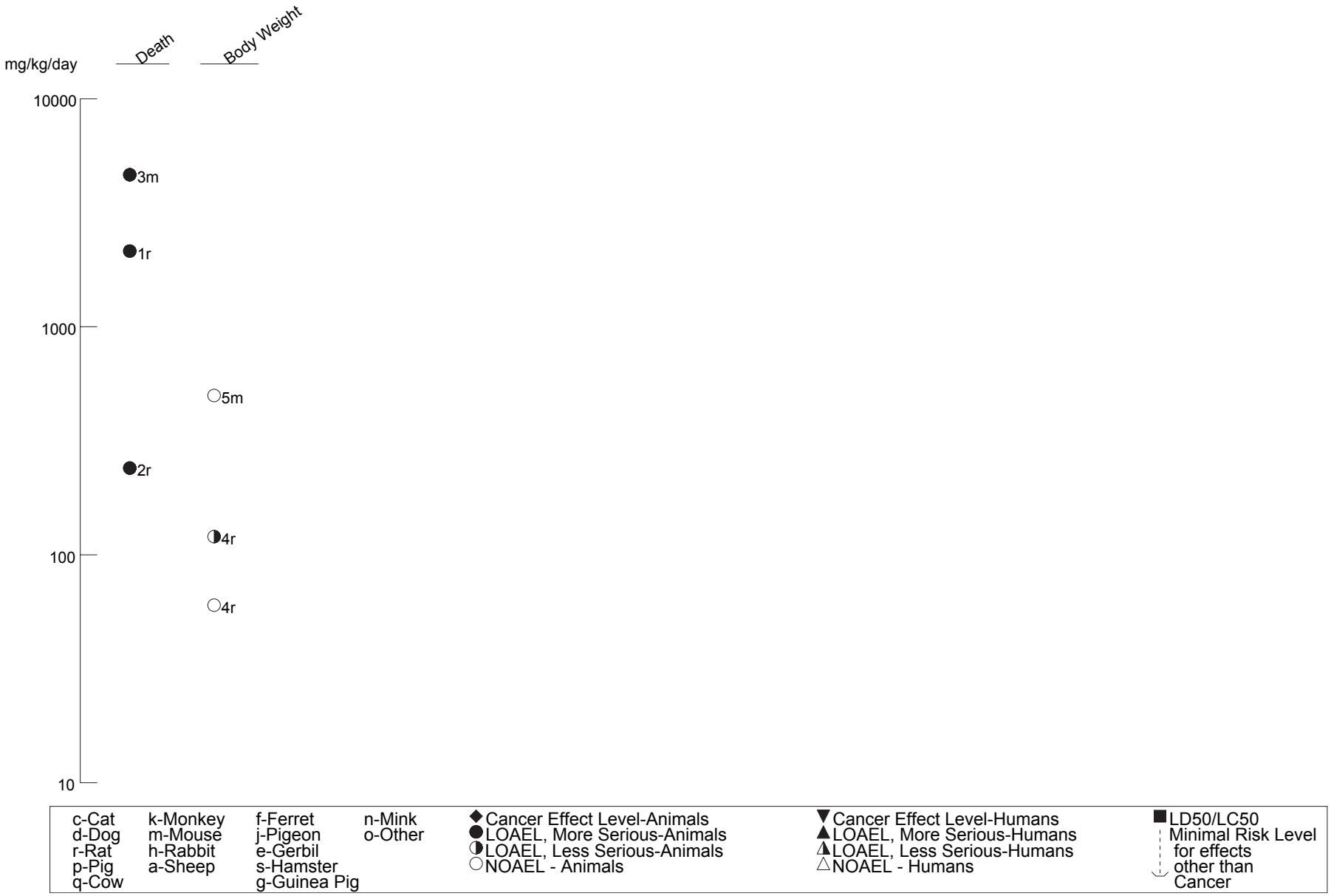
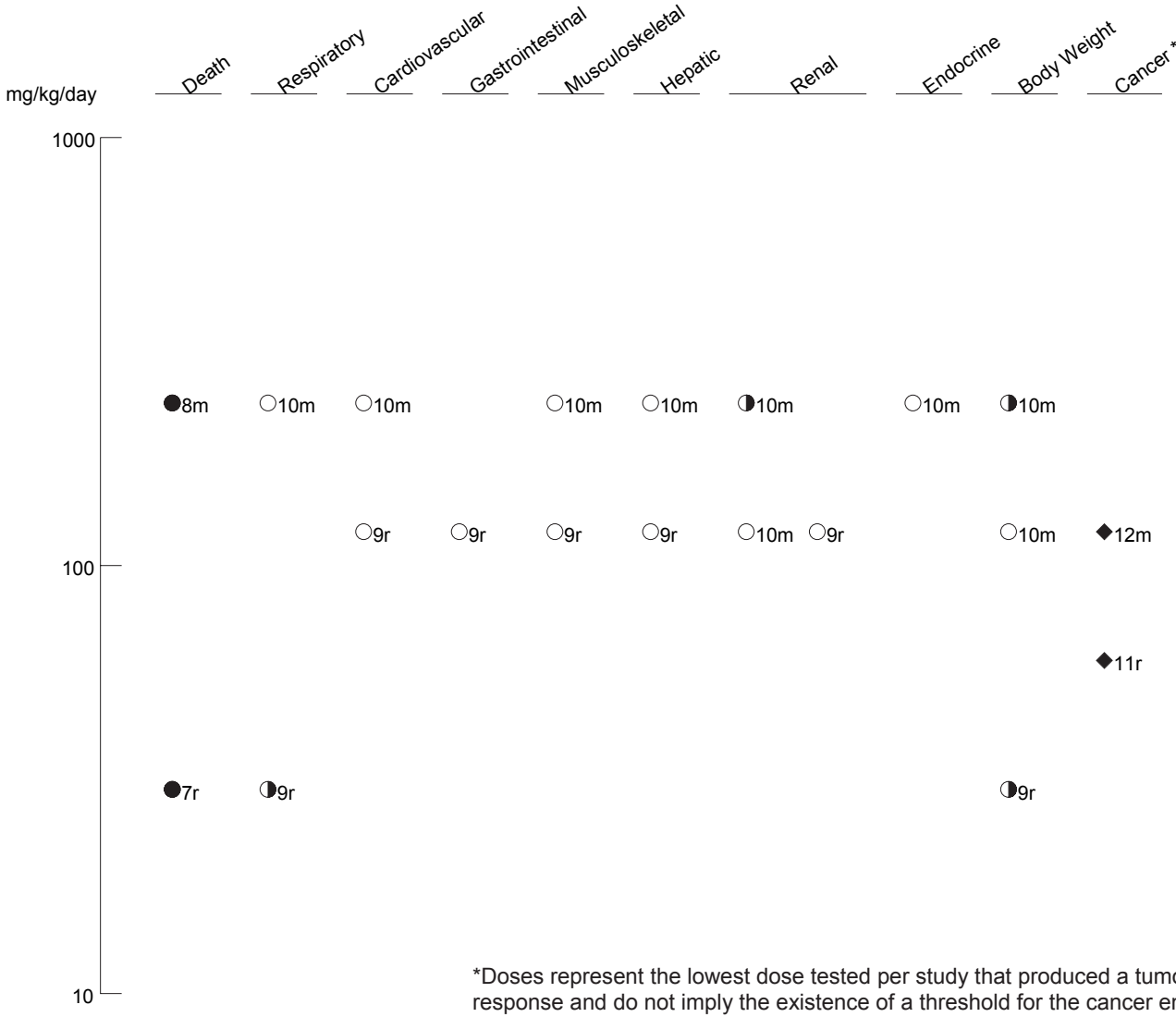


Figure 3-3 Levels of Significant Exposure to Toluene Diisocyanate - Oral *(Continued)*

Intermediate (15-364 days)

Figure 3-3 Levels of Significant Exposure to Toluene Diisocyanate - Oral *(Continued)*

Chronic (≥365 days)



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

c-Cat
d-Dog
r-Rat
p-Pig
q-Cow

k-Monkey
m-Mouse
h-Rabbit
a-Sheep

f-Ferret
j-Pigeon
e-Gerbil
s-Hamster
g-Guinea Pig

n-Mink
o-Other

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

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

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

In an acute lethality study using five animals/sex/dose, two of five male F344 rats given a single gavage dose of 2,150 mg/kg (the lowest dose tested) commercial-grade TDI in corn oil died on days 5 and 9 of the observation period (NTP 1986). No females died at this dose, but at 3,160 mg/kg, two of five female rats died. Dose-related increases in mortality were observed in both sexes at higher doses. There were no untreated controls in this study. In a 14-day gavage study of rats by NTP (1986), there were clear dose-related increases in mortality at doses ≥500 mg/kg/day. A second study by this group reported that one male and one female each in the 30 and 240 mg/kg/day dose groups, and one female in the 500 mg/kg/day group died prematurely, but there were no deaths at 60 or 120 mg/kg/day. Taken together with the first study, these data suggest a severe LOAEL of 240 mg/kg/day for death in rats (NTP 1986).

In the acute lethality study of mice performed by NTP (1986), a dose-related increase in mortality was observed at doses ≥ 4,640 mg/kg. At 4,640 mg/kg, one of five female mice and four of five male mice died between days 2 and 8 of the observation period. No deaths occurred at doses of 2,150 mg/kg (males only were exposed to this dose) or 3,160 mg/kg (males and females). As with the rat study, there were no untreated controls in this study. In 14-day gavage studies of commercial-grade TDI in mice (NTP 1986), all animals died in the first study using doses 500 mg/kg/day. A second study using doses of 30–500 mg/kg/day was performed, and deaths of one to two male mice per group were seen at all doses. Two females died at 240 mg/kg/day, but there were no female deaths at any other dose. An effect level for death is difficult to ascertain from this study due to the lack of a dose-response relationship in the male deaths at doses between 30 and 500 mg/kg/day in the second study and the deaths of all animals at ≥500 mg/kg/day in the first study.

Intermediate-duration (13 weeks, 5 days/week) gavage studies of commercial-grade TDI in rats and mice were performed by NTP (1986). In both species, the 13-week studies were repeated due to mortality in the first study. As with the 14-day studies, deaths occurred at various doses without a clear dose-response relationship. The study authors considered a single female rat death at 240 mg/kg/day, and deaths of 1/10 and 2/10 female mice exposed to 120 and 240 mg/kg/day (respectively), to be treatment-related (NTP 1986).

Chronic (2-year) exposure to gavage doses ≥30 mg/kg/day TDI for 5 days/ week significantly decreased survival of F344 rats (NTP 1986). At termination, survival of male rats was 36/50, 14/50, and 8/50 at 0, 30, and 60 mg/kg/day, respectively; survival of female rats was 36/50, 19/50, and 6/50 at 0, 60, and 120 mg/kg/day, respectively. In the chronic study of mice (NTP 1986), survival of high-dose (240 mg/kg/day) male mice was significantly lower than controls; at termination, 46/50 controls, 40/50 in

the 120 mg/kg/day group, and 26/50 in the 240 mg/kg/day group remained. Survival of female mice was not diminished by treatment.

3.2.2.2 Systemic Effects

No information was located regarding systemic effects in humans following oral exposure to TDI.

Respiratory Effects. No information on respiratory effects of acute-duration oral exposure to TDI in animals was located. In rats exposed via gavage to commercial-grade TDI for 13 weeks (5 days/week), mucoid bronchopneumonia was reported in one of 2 male rats that received 120 mg/kg/day and died prematurely; this effect was also seen in 8/10 males and 2/10 females exposed to 240 mg/kg/day (NTP 1986). Histopathology examination was not performed on other rats in the 120 mg/kg/day group or in any rats of the lower dose groups; thus, an effect level cannot be determined for this end point. In the 13-week study of mice, few results of the histopathology examination of high dose animals were reported, but results that were reported did not indicate lesions of the respiratory tract at 240 mg/kg/day.

When F344 rats were exposed via gavage to commercial-grade TDI on 5 days/week for 2 years, acute bronchopneumonia was seen in both sexes (NTP 1986). The incidences of bronchopneumonia in male rats were 2/50, 6/50, and 14/50 (control, 30, and 60 mg/kg/day groups, respectively) and incidences in female rats were 1/50, 10/50, and 25/49 (control, 60, and 120 mg/kg/day groups, respectively). In contrast, B6C3F1 mice exhibited no respiratory effects when exposed for 2 years to commercial-grade TDI doses up to 120 mg/kg/day in females and 240 mg/kg/day in males (NTP 1986).

Cardiovascular Effects. No histological alterations were observed in the hearts of rats or mice administered doses up to 120 or 240 mg/kg/day commercial-grade TDI 5 days/week for 2 years (NTP 1986).

Gastrointestinal Effects. Chronic gavage studies with TDI in rats and mice did not result in gastrointestinal lesions (NTP 1986).

Hematological Effects. No information was located regarding hematological effects in animals following oral exposure to TDI.

Musculoskeletal Effects. No histological alterations were observed in the musculoskeletal system of rats or mice administered doses up to 120 or 240 mg/kg/day commercial-grade TDI 5 days/week for 2 years (NTP 1986).

Hepatic Effects. No non-neoplastic lesions resulted from a 2-year administration of commercial-grade TDI to rats and mice (NTP 1986).

Renal Effects. No histological alterations were observed in the kidneys of administered doses up to 120 mg/kg/day commercial-grade TDI 5 days/week for 2 years (NTP 1986). An increased incidence of cytomegaly of tubular epithelium was observed in male mice administered 240 mg/kg/day commercial-grade TDI 5 days/week for 2 years (NTP 1986); no alterations were observed in male or female mice exposed to 120 mg/kg/day.

Dermal Effects. Administration of commercial-grade TDI for 2 years did not result in dermal lesions in rats or mice (NTP 1986).

Ocular Effects. Administration of commercial-grade TDI for 2 years did not result in ocular lesions in rats or mice (NTP 1986).

Body Weight Effects. Decreases in body weight gain were observed in male and female rats administered via gavage ≥30 mg/kg/day commercial-grade TDI 5 days/week for 2 years (NTP 1986). A similar exposure of mice resulted in decreases in body weight gain at 240 mg/kg/day (males only); no alterations in weight gain were observed in male or female mice at 120 mg/kg/day (NTP 1986).

3.2.2.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological and lymphoreticular effects in humans or animals following oral exposure to TDI.

3.2.2.4 Neurological Effects

No information was located regarding neurological effects in humans or animals following oral exposure to TDI.

3.2.2.5 Reproductive Effects

No information was located regarding reproductive effects in humans or animals following oral exposure to TDI.

3.2.2.6 Developmental Effects

No information was located regarding developmental effects in humans or animals following oral exposure to TDI.

3.2.2.7 Cancer

Data on cancer effects of diisocyanates in humans and animals orally exposed to diisocyanates are limited to bioassays in rats and mice exposed to commercial-grade TDI via gavage (NTP 1986). Based on the bioassays, NTP (1986) concluded that there was clear evidence that commercial-grade TDI in corn oil was carcinogenic to female mice and to rats of both sexes. Tumor types occurring at increased incidences in the exposed rats included subcutaneous fibromas and fibrosarcomas, pancreatic acinar cell adenomas and islet cell adenomas, mammary gland fibroadenomas, and neoplastic nodules of the liver. In exposed female mice, the following tumor types occurred at increased incidences: hemangiomas or hemangiosarcomas and hepatocellular adenomas. The findings of the study are limited by reduced survival in rats and high dose male mice, as well as instability of the test material in the vehicle.

NTP (1986) administered commercial-grade TDI in corn oil via gavage to groups of 50/sex F344 rats and B6C3F1 mice on 5 days/week for 104 weeks. Doses were 0, 60, or 120 mg/kg/day in female rats and mice; 0, 30, or 60 mg/kg/day in male rats; and 0, 120, or 240 mg/kg/day in male mice. Analysis of the administered material indicated that the TDI had reacted with corn oil, yielding actual gavage doses 77–90% of nominal doses. In rats, survival was significantly lower than controls in all exposed groups; NTP (1986) concluded that the maximum tolerated dose had been exceeded. Statistically significant increases in the incidence of neoplasia were observed in both male and female rats; the data are shown in Table 3-6. Increased incidences of subcutaneous tissue fibromas or fibrosarcomas and pancreatic acinar cell adenomas occurred in male rats. In female rats, mammary gland fibroadenomas, pancreatic islet cell adenomas, and neoplastic nodules of the liver were observed; mammary gland and pancreatic tumor incidences were significantly higher than controls at both doses, while the incidence of hepatic neoplastic nodules was significantly increased only at the high dose in female rats (NTP 1986). Survival of high-dose male mice was significantly lower than controls, but survival of female mice was not diminished by

treatment (NTP 1986). Statistically significant increases in the incidence of neoplasia were observed only in high dose female mice, and included hemangiosarcomas or hemangiomas and hepatocellular adenomas or carcinomas (Table 3-6). NTP (Dieter et al. 1990) noted that the liver, mammary gland, and subcutaneous tissue tumors observed in rats and hemangiomas and liver tumors observed in mice were the same types of tumors observed in rats and mice exposed to 2,4-diaminotoluene, a known carcinogen. Dieter et al. (1990) suggested that the carcinogenic activity observed in the NTP (1986) study could be attributed to the metabolism of 2,4-TDI to products identical to those of 2,4-diaminotoluene metabolism. An industry-sponsored statistical analysis of the results of the NTP TDI study and NTP 2,4-diamino-toluene study concluded that hydrolyzation of 5% of the TDI dose to form 2,4-aminotoluene could explain carcinogenic responses observed in the NTP TDI study (Sielken et al. 2012).

3.2.3 Dermal Exposure

3.2.3.1 Death

No information was located regarding deaths in humans or animals following dermal exposure to TDI or MDI.

3.2.3.2 Systemic Effects

No information was located regarding systemic effects in humans or animals following dermal exposure to TDI. Data on the dermal toxicity of MDI are limited to a human study that reported respiratory effects.

Respiratory Effects.

MDI. Workers at a newly established wood products facility with no prior exposure to MDI were asked to complete symptom questionnaires prior to beginning work and 2, 9, 14, and 20 months after production began (Petsonk et al. 2000; Wang and Petsonk 2004); the workers were exposed to liquid MDI resin.

Asthma-like symptoms were reported by 15 of the 56 workers with the highest potential for exposure to liquid MDI and prepolymer, as compared to 0 of 43 workers with the lowest exposure potential. MDI-exposed workers had significantly increased odds of dyspnea with wheezing, dyspnea or cough at rest, chest tightness, and phlegm after adjusting for age, smoking, and wood dust exposure (Wang and Petsonk 2004). There were no increases in the prevalence of eye or nasal symptoms. MDI exposure was likely via the inhalation and dermal routes of exposure. The workers wore respirators; however, the incidence

Table 3-6. Tumor Incidences in Rats and Mice Exposed to Commercial-Grade Toluene Diisocyanate for 2 Years by Gavage[a,b]

	Control	30 mg/kg/day	60 mg/kg/day	120 mg/kg/day
Male rats				
Subcutaneous fibroma or fibrosarcoma	3/50 (6%)	6/50[b] (12%)	12/50[b] (24%)	Not tested
Pancreatic acinar cell adenoma	1/47 (2%)	3/47 (6%)	7/49[b] (14%)	Not tested
Pancreatic islet cell adenoma or carcinoma	1/47 (2%)	0/47 (0%)	4/49[b] (8%)	Not tested
Female rats				
Subcutaneous fibroma or fibrosarcoma	2/50 (4%)	Not tested	1/50 (2%)	5/50[b] (10%)
Mammary gland tumors	17/50 (34%)	Not tested	25/50[b] (50%)	21/50[b] (42%)
Pancreatic islet cell adenoma	0/50 (0%)	Not tested	6/49[b] (12%)	2/47 (4%)
Hepatic neoplastic nodules	3/50 (6%)	Not tested	8/50[b] (16%)	8/48[b] (17%)
Female mice				
Hemangioma or hemangiosarcoma	0/50 (0%)	Not tested	1/50 (2%)	5/50[b] (10%)
Hepatocellular adenoma or carcinoma	4/50 (8%)	Not tested	5/50 (10%)	15/50[b] (30%)

[a]Data are presented as the number of animals with tumor per total number of animals examined in each exposure group (percentages in parentheses).
[b]Significantly different from control by either life table or incidental tumor test or both, $p<0.05$.

Source: NTP 1986

of asthma symptoms was significantly higher among those who reported removing their respirator during work. The incidence of asthma symptoms was also significantly higher in workers reporting skin or clothing MDI staining (Petsonk et al. 2000).

3.2.3.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological and lymphoreticular effects in humans following dermal exposure to TDI or MDI.

In mice, dermal exposure to 2,4-TDI or MDI followed by an oral challenge dose resulted in airway hyperreactivity, lung tissue hyperreactivity, and increases in serum IgE levels (Pollaris et al. 2016). However, no evidence of cross-reactivity was observed in mice exposed to 2,4-TDI and challenged with MDI or exposed to MDI and challenged with 2,4-TDI.

3.2.3.4 Neurological Effects

No information was located regarding neurological effects in humans or animals following dermal exposure to TDI or MDI.

3.2.3.5 Reproductive Effects

No information was located regarding reproductive effects in humans or animals following dermal exposure to TDI or MDI.

3.2.3.6 Developmental Effects

No information was located regarding developmental effects in humans or animals following dermal exposure to TDI or MDI.

3.2.3.7 Cancer

No information was located regarding cancer in humans or animals following dermal exposure to TDI or MDI.

3.3 GENOTOXICITY

TDI. Results of *in vitro* genotoxicity testing of TDI are shown in Table 3-7. 2,4-TDI, 2,6-TDI, and the commercial-grade mixture (80:20 mixture of 2,4- and 2,6-TDI) have all been tested for mutagenicity in various strains of *Salmonella typhimurium* (Anderson and Styles 1978; Anderson et al. 1980; NTP 1986; Seel et al. 1999; Zeiger et al. 1987). All of the studies have shown negative results in the absence of metabolic activation.

All of the studies other than Seel et al. (1999) used dimethylsulfoxide (DMSO) as a solvent for the test compounds, and these studies suggested that each of the individual isomers and the mixture was mutagenic with metabolic activation in at least one strain of *S. typhimurium* (NTP 1986; Zeiger et al. 1987). Seel et al. (1999) showed that 2,4-TDI is not stable in DMSO (a hygroscopic solvent that increases reaction of TDI with water), and that use of this solvent yielded a variety of degradation products, including 2,4-TDA (a known mutagen), in the reaction medium. To assess the role of toluene diamines in the observed responses of TDI in these tests, Seel et al. (1999) conducted parallel mutagenicity tests using DMSO and ethylene glycol dimethylether (EGDE) as solvents and quantifying levels of TDI and TDA in the first 45 seconds after the test was started. These tests showed that 2,6-TDI was relatively more stable in EGDE than in DMSO; when DMSO was used as a solvent, only 12.3% of dose of 2,6-TDI remained at the start of mutagenicity testing, while 9.1% of the dose existed as 2,6-TDA (other reaction products were not analyzed). In contrast, when EGDE was used, 99.5% of the dose existed as 2,6-TDI at the start of testing, with only 0.5% as 2,6-TDA (Seel et al. 1999). Analyses over time showed formation of TDA in mixtures using either DMSO or EGDE; levels of TDA were lower when EGDE was used, but not substantially lower after the first 45 seconds (2,6-TDA was 5.6% of the TDI dose in the EGDE mixture, compared with 8.3% of the TDI dose in the DMSO mixture). These experiments indicated that mutagenicity testing of TDI using DMSO as a solvent yields unreliable results due to the conversion of TDI to TDA prior to plating.

In mutagenicity tests using EGDE as the solvent (Seel et al. 1999) and with metabolic activation, all three test materials tested positive in TA98 and TA100 and all three tested negative in TA1535. 2,4-TDI was also positive in strain TA1537, and the 80:20 mixture was weakly positive in this strain, while 2,6-TDI was negative. The authors attributed the positive and weakly positive results to the TDA formed over time even when EGDE was used.

Table 3-7. Genotoxicity of TDI and MDI *In Vitro*

Species (test system)	End point	Results		Purity	Vehicle	Reference
		With activation	Without activation			
2,6-TDI						
Prokaryotic organisms:						
Salmonella typhimurium (TA100, TA98)	Gene mutation	+	–	94%	DMSO	NTP 1986; Zeiger et al. 1987
S. typhimurium (TA98)	Gene mutation	+	–	NR	EGDE	Seel et al. 1999
S. typhimurium (TA1535, TA1537[a])	Gene mutation	–	–	94%	DMSO	NTP 1986; Zeiger et al. 1987
S. typhimurium (TA1537)	Gene mutation	–	–	NR	EGDE	Seel et al. 1999
Mammalian cells:						
L5178Y mouse lymphoma cells	Gene mutation	+	+	NR	DMSO	McGregor et al. 1991
Chinese hamster ovary cells	Sister chromatid exchange	–	+	99%	DMSO	Gulati et al. 1989
Chinese hamster ovary cells	Chromosomal aberrations	–	+	99%	DMSO	Gulati et al. 1989
2,4-TDI						
Prokaryotic organisms:						
S. typhimurium (TA100, TA98)	Gene mutation	+	–	99%	DMSO	Zeiger et al. 1987
S. typhimurium (TA1535, TA97)	Gene mutation	(+/–)	–	99%	DMSO	Zeiger et al. 1987
S. typhimurium (TA98, TA100, TA1535, TA1538)	Gene mutation	–	NT	NR	DMSO	Anderson and Styles 1978
S. typhimurium (TA1537, TA98)	Gene mutation	+	–	NR	EGDE	Seel et al. 1999
Mammalian cells:						
L5178Y mouse lymphoma cells	Gene mutation	+	+	NR	DMSO	McGregor et al. 1991
Chinese hamster ovary cells	Sister chromatid exchange	–	(+/–)	94%	DMSO	Gulati et al. 1989
Chinese hamster ovary cells	Chromosomal aberrations	–	–	94%	DMSO	Gulati et al. 1989

Table 3-7. Genotoxicity of TDI and MDI *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Purity	Vehicle	Reference
Commercial-grade 2,4- and 2,6-TDI (80:20 mixture)						
Prokaryotic organisms:						
S. typhimurium (TA100, TA98)	Gene mutation	+	–	Commercial grade	DMSO	NTP 1986; Zeiger et al. 1987
S. typhimurium (TA100, TA98, TA1537)	Gene mutation	+	–	Commercial grade	EGDE	Seel et al. 1999
S. typhimurium (TA100, TA98, TA1538)	Gene mutation	+	–	Commercial grade	DMSO	Anderson et al. 1980
S. typhimurium (TA1537)	Gene mutation	–	–	Commercial grade	DMSO	Anderson et al. 1980
S. typhimurium (TA1535, TA1537)	Gene mutation	–	–	Commercial grade	DMSO	NTP 1986; Zeiger et al. 1987
Mammalian cells:						
F-344 rat hepatocyte primary cultures	Unscheduled DNA synthesis	–	–	NR	DMSO	Shaddock et al. 1990
4,4'-MDI (monomer)						
Prokaryotic organisms:						
S. typhimurium (TA100, TA98)	Gene mutation	+	–	NR	DMSO	Herbold et al. 1998
S. typhimurium (TA100, TA98)	Gene mutation	+	–	98%	DMSO	Shimizu et al. 1985
S. typhimurium (TA100, TA98)	Gene mutation	+	–	Commercial grade	DMSO	Anderson et al. 1980
S. typhimurium (TA100, TA98)	Gene mutation	–	–	NR	EGDE	Herbold et al. 1998
S. typhimurium (TA1535, TA1537, TA1538)	Gene mutation	–	–	98%	DMSO	Shimizu et al. 1985
S. typhimurium (TA1535, TA1537)	Gene mutation	–	–	NR	DMSO, EGDE	Herbold et al. 1998
S. typhimurium (TA1537)	Gene mutation	–	–	Commercial grade	DMSO	Anderson et al. 1980
Mammalian cells:						
Human lung epithelial cells (A549)	DNA double-strand breaks	NT	–(c)	NR	EGDE	Vock et al. 1998

Table 3-7. Genotoxicity of TDI and MDI *In Vitro*

Species (test system)	End point	Results		Purity	Vehicle	Reference
		With activation	Without activation			
2,4-MDI						
S. typhimurium (TA98, TA1538)	Gene mutation	+	–	NR	DMSO	Herbold et al. 1998
S. typhimurium (TA98, TA1538)	Gene mutation	–	–	NR	EGDE	Herbold et al. 1998
S. typhimurium (TA100)	Gene mutation	–	–	NR	DMSO, EGDE	Herbold et al. 1998
Mixture of isomers monomeric MDI (4,4'-, 2,4'-, and 2,2'-)						
S. typhimurium (TA100, TA98)	Gene mutation	+	–	NR	DMSO	Herbold et al. 1998
S. typhimurium (TA100, TA98, TA1535, TA1537)	Gene mutation	–	–	NR	EGDE	Herbold et al. 1998
S. typhimurium (TA1535, TA1537)	Gene mutation	–	–	NR	DMSO	Herbold et al. 1998
Polymeric MDI						
S. typhimurium (TA100, TA98)	Gene mutation	+	–	NR	DMSO	Herbold et al. 1998
S. typhimurium (TA100, TA98, TA1535, TA1537)	Gene mutation	–	–	NR	EGDE	Herbold et al. 1998
S. typhimurium (TA1535, TA1537)	Gene mutation	–	–	NR	DMSO	Herbold et al. 1998

[a]Zeiger et al. (1987) incorrectly show the tested strain as TA97; the data shown are identical to those shown for TA1537 in the original report (NTP 1986).

– = negative result; +/– = mixed results; + = positive result; –(c); positive only at cytotoxic concentrations; DMSO = dimethylsulfoxide; DNA = deoxyribonucleic acid; EGDE = ethylene glycol dimethylether; MDI = methylenediphenyl diisocyanate; NR = not reported; NT = not tested; TDI = toluene diisocyanate

Experiments conducted by Seel et al. (1999) also included using two different S9 microsome quantities: 10 and 30%. The authors observed that the mutagenic responses were slightly diminished in the tests with higher S9 content, and postulated that the higher protein content in the 30% S9 mix provided alternative substrates for TDI reaction, yielding relatively lower amounts of the mutagenic TDA degradation products.

TDI has also been tested in mammalian cell systems for mutagenicity (McGregor et al. 1991), chromosomal aberrations, and sister chromatid exchanges (Gulati et al. 1989), as well as unscheduled DNA synthesis (Shaddock et al. 1990), as shown in Table 3-7. All of the tests used DMSO as the test material solvent.

Only commercial-grade TDI has been tested for *in vivo* genotoxicity (see Table 3-8); data on the individual isomers are not available. In workers exposed occupationally to TDI (0.007–0.016 mg/m^3) during plastic production, significantly increased numbers of sister chromatid exchanges, micronuclei, and structural chromosomal aberrations were observed in peripheral blood lymphocytes, when compared with unexposed persons from the same geographic area (Bilban et al. 2004). The study did not adjust for the statistically significant differences in average age and smoking index (number of cigarettes smoked per day per years of smoking) between the exposed and unexposed groups; the exposed group was older and had a higher smoking index. In a controlled exposure experiment, Marczynski et al. (2005) compared the frequency of DNA strand breaks in lymphocytes before and after exposure of 5 workers with prior diisocyanate exposure and airway symptoms and 10 subjects without prior exposure but with asthma or bronchial hyperresponsiveness. The subjects were exposed to industrial-grade TDI (80:20 mixture of 2,4- and 2,6-TDI) in the following sequence: 30 minutes at 5 ppb, 30 minutes at 10 ppb, 90-minute break, 30 minutes at 20 ppb, 90-minute break, and ending with 30 minutes at 30 ppb. Blood was sampled for use in the comet assay before as well as 30 minutes and 19 hours after the end of exposure. Blood was also collected at the same time points from a group of 10 healthy subjects who were not subjected to any exposure. Mean values of the olive tail moment before and after exposure did not differ significantly, nor were there significant differences between the groups. In workers exposed to isocyanates, primarily TDI and MDI (mean TDI levels ranged from <1 to 60 µg/m^3), and several tertiary amines, no significant increases in chromosomal aberrations, sister chromatid exchanges, or micronuclei frequency in peripheral lymphocytes were observed, as compared to a referent group (Holmen et al. 1988). Exposing *S. typhimurium* T98 or *E. coli* WP2 uvrA to urinary samples from the exposed workers did not result in increases in mutagenic activity.

Table 3-8. Genotoxicity of TDI and MDI *In Vivo*

Species (test system)	End point	Results	Purity or grade	Route of exposure	Reference
2,6-TDI					
No data					
2,4-TDI					
No data					
Commercial-grade 2,4- and 2,6-TDI (80:20 mixture)					
Humans					
Peripheral blood lymphocytes	Micronuclei	+	NA	Inhalation	Bilban 2004
Peripheral blood lymphocytes	Structural chromosomal aberrations	+	NA	Inhalation	Bilban 2004
Peripheral blood lymphocytes	Sister chromatid exchange	+	NA	Inhalation	Bilban 2004
Peripheral blood lymphocytes	DNA strand breaks	–	Industrial grade	Inhalation	Marczynski et al. 2005
Non-human mammals					
Mouse bone marrow	Micronuclei	–	Production grade	Inhalation	Loeser et al. 1983
Mouse bone marrow	Micronucleated PCEs	–	NR	Inhalation	Lindberg et al. 2011
Mouse bone marrow	Chromosomal aberrations	+	95%	Inhalation	Ji et al. 2008
Mouse bone marrow	Sister chromatid exchange	+	95%	Inhalation	Ji et al. 2008
Mouse peripheral blood	Micronucleated PCEs	–	NR	Inhalation	Lindberg et al. 2011
Rat bone marrow	Micronuclei	–	Production grade	inhalation	Loeser et al. 1983
Non-mammalian systems					
Drosophila melanogaster post-meiotic and meiotic germ cells	Sex-linked recessive lethal mutation	+	99%	Feeding (ethanol vehicle)	Foureman et al. 1994
D. melanogaster post-meiotic and meiotic germ cells	Translocation	+	99%	Feeding (ethanol vehicle)	Foureman et al. 1994

Table 3-8. Genotoxicity of TDI and MDI *In Vivo*

Species (test system)	End point	Results	Purity or grade	Route of exposure	Reference
4,4'-MDI (monomer)					
Peripheral blood lymphocytes	DNA strand breaks	–	Industrial grade: 60% methylene-diphenyl diisocyanate, 30% triiso-cyanates, 10% diisocyanates	Inhalation	Marczynski et al. 2005
Mouse bone marrow	Micronucleated PCEs	–	98%	Inhalation	Lindberg et al. 2011
Mouse peripheral blood	Micronucleated PCEs	–	98%	Inhalation	Lindberg et al. 2011
Rat bone marrow	Micronucleated PCEs	+		Inhalation	Zhong and Siegel 2000
Rat bone marrow	Micronuclei	–	99%	Inhalation	Pauluhn et al. 2001
Rat epidermis and liver	DNA adduct formation	–	NR	Dermal	Vock and Lutz 1997
Rat epidermis	DNA adduct formation	–	NR	Dermal	Vock et al. 1995

– = negative result; + = positive result; (+/–) = mixed results; DNA = deoxyribonucleic acid; MDI = methylenediphenyl diisocyanate; NA = not applicable; NR = not reported; NT = not tested; PCE = polychromated erythrocyte; TDI = toluene diisocyanate

Lindberg et al. (2011) observed no increase in the frequency of micronucleated polychromatic erythrocytes (PCEs) in mouse bone marrow or peripheral blood after five daily 1-hour periods of exposure to TDI vapor (63% 2,4-TDI and 37% 2,6-TDI) at concentrations up to 2.4 mg/m^3 (0.34 ppm). Similarly, Loeser et al. (1983) did not observe an increase in micronucleated erythrocytes in bone marrow of rats or mice exposed for 4 weeks to vapor concentrations of 0, 0.05, or 0.15 ppm, 6 hours/day for 5 days/week. Ji et al. (2008) reported a significant increase in the frequencies of chromosomal aberrations and sister chromatid exchanges in bone marrow of mice exposed for 4 hours/day on 14 consecutive days to TDI vapor (composition not specified, but reported as 95% pure) at a concentration characterized as one-fourth of the LC_{50} (no other exposure details were provided). However, given the lack of study details, especially the absence of information on exposure concentration, the results reported by Ji et al. (2008) cannot be evaluated in the context of the other available data.

In *in vivo* tests of sex-linked recessive lethal mutation and translocation using male *Drosophila* exposed by feeding, commercial-grade TDI (mixture of 2,4- and 2,6- isomers of unknown composition, administered in ethanol) yielded positive results (Foureman et al. 1994).

MDI. Studies of the *in vitro* genotoxicity of MDI are shown in Table 3-7. As was seen with experiments on TDI, stability testing of MDI in DMSO showed diminished levels of free MDI as a function of time in the solvent (Herbold et al. 1998). However, in contrast to TDI, the rate of MDI degradation in both DMSO and EGDE was much slower. In fact, MDI was stable in EGDE; even in the presence of 12.78 mM water, <1% of the tested mass of MDI (tested as 4,4'-MDI, monomeric MDI isomers, and polymeric MDI) had degraded after 4 hours (Herbold et al. 1998). Consistent with its greater stability in EGDE, MDI was uniformly negative in mutagenicity testing using this solvent (Herbold et al. 1998), while positive results (in TA98 and TA100 for monomeric and polymeric MDI and 4,4'-MDI and in TA1538 for 2,4-MDI) were observed when DMSO was used as the solvent (Anderson et al. 1980; Herbold et al. 1998; Shimizu et al. 1985).

Only 4,4'-MDI has been tested for genotoxicity in mammalian cells; Vock et al. (1998) observed double-strand DNA breaks in human lung epithelial cells (A549) at 4,4'-MDI concentrations (in EGDE) that were cytotoxic. The authors noted that cytotoxicity in the test system was exacerbated both by the slight toxicity of the EGDE solvent and by the lack of nutrients and growth factors in the test solution (phosphate-buffered saline was used instead of growth medium to minimize reaction between MDI and

medium constituents like proteins). The study authors concluded that the observed DNA damage was a function of cytotoxicity rather than direct genotoxicity.

Likewise, 4,4'-MDI is the only isomer or composition that has been tested for genotoxicity in *in vivo* systems (Table 3-8). Marczynski et al. (2005) assessed the potential of MDI to induce DNA strand breaks. MDI workers (n=25) and controls (n=10) were exposed to 4,4'-MDI in the same sequences as the TDI study: 30-minute exposure to 5 ppb, 30-minute exposure to 10 ppb, 90-minute break, 30-minute exposure to 20 ppb, 90-minute break, and 30-minute exposure to 30 ppb. 4,4'-MDI exposure did not significantly increase DNA strand breaks, as assessed using olive tail moment comet assay. Lindberg et al. (2011) observed no increase in the frequency of micronucleated PCEs in mouse bone marrow or peripheral blood after five daily 1-hour periods of exposure to 4,4'-MDI aerosol at concentrations up to 23.3 mg/m^3. Zhong and Siegel (2000) reported a concentration-dependent increase in the frequency of micronucleated PCEs in the bone marrow of male Brown-Norway rats 7 days following exposure to 4,4'-MDI. The rats were exposed for 3 weeks, 1 hour/week to concentrations of 7 and 113 mg/m^3 4,4'-MDI aerosol. Pauluhn et al. (2001) also exposed male Brown-Norway rats for 1 hour/week for 3 weeks at concentrations up to 118 mg/m^3 of 4,4'-MDI aerosol. Pauluhn et al. (2001) sacrificed the rats on post-exposure days 1, 2, and 7; no increase in micronucleated PCEs was seen at any time point. Vock and colleagues (Vock and Lutz 1997; Vock et al. 1995) observed minimal to no induction of isocyanate-DNA adducts (assessed by ^{32}P postlabelling) in the skin liver, kidney, lung, and bladder of female Wistar rats exposed to 6.9–9 mg 4,4'-MDI in acetone via topical application.

3.4 TOXICOKINETICS

Both TDI and MDI combine readily with biological macromolecules including hemoglobin, albumin, and others. As a consequence of their reactivity, these compounds or their reaction products are often found at higher concentrations at the site of entry into the body early in exposure, and may continue to be distributed from the site of entry long after exposure has terminated.

Many studies in humans and laboratory animals use levels of diamines (TDA or methylenediphenyl diamine [MDA]) as a biomarker to evaluate TDI and MDI toxicokinetic properties. Most studies are not measuring free TDA or MDA levels that are the result of TDI or MDI metabolism. Rather, the studies are treating the plasma and urine samples with acids or bases to hydrolyze the diisocyanate-protein or diamine-protein conjugates and acetylated diamines, resulting in the formation of free diamine (Sennbro et al. 2004; Sepai et al. 1995).

TDI is absorbed after human exposure, but available data are not adequate to permit estimation of the rate or extent of absorption. In rats, absorption of inhaled 2,4-TDI was estimated to be between 61 and 90% (Timchalk et al. 1994). One study in rats exposed to monomeric 4,4'-MDI as an aerosol estimated that 32% of an inhaled dose of 0.078 mg was systemically available (Gledhill et al. 2005). Limited data are available on the oral absorption of TDI or MDI. Following gavage administration of 2,4-TDI, 12–20% of the dose was absorbed (Timchalk et al. 1994); no data on the oral absorption of MDI are available. There is evidence that both TDI and MDI are absorbed across the skin to some extent, but the available data do not provide clear estimates of the rate or extent of absorption.

Once absorbed into the body, TDI is bound to macromolecules, forming adducts with hemoglobin, albumin, glutathione, and other macromolecules. The binding of TDI to glutathione appears to be reversible (Day et al. 1997), and may represent a mechanism by which TDI is transported between tissues. After inhalation (Kennedy et al. 1994; Timchalk et al. 1994) and gavage administration (Timchalk et al. 1994) exposure of rats to radiolabeled TDI, radioactivity was detected in a number of tissues, albeit at low levels. Systemic distribution of low levels of radioactivity has also been observed after inhalation (Gledhill et al. 2005) and dermal exposure (Vock et al. 1997) of rats to radiolabeled MDI.

The metabolic fate of TDI depends on the exposure route. After oral exposure, TDI is hydrolyzed in the gastrointestinal tract to TDA, and subsequently either absorbed and metabolized further or reacted with unhydrolyzed TDI to form polyurea polymers that pass unabsorbed through the gastrointestinal tract. However, after inhalation exposure, the primary fate of TDI appears to be conjugation reactions; little to no TDI is hydrolyzed to TDA. Little information on the metabolism of MDI was located; the single available study (Gledhill et al. 2005) indicated that after inhalation exposure of rats to MDI aerosol, the primary metabolites in the urine and bile were N-acetylated and N-acetylated hydroxylated products of MDI, and the primary product in feces is believed to be mixed molecular weight polyureas resulting from spontaneous reaction of MDI.

In humans exposed experimentally, TDA levels in hydrolyzed urine exhibits a biphasic pattern, with an initial rapid phase followed by a slower phase. The primary route of TDI elimination after inhalation or oral exposure of rats is via the feces, which may include material absorbed and excreted via the bile. Data on the elimination of MDI are limited. Like TDI, MDI is excreted primarily in the feces of rats after inhalation exposure, and there is evidence for biliary excretion of MDI. No studies of MDI elimination after oral exposure were located in the available literature.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

TDI. Two studies reporting urinary concentrations of diisocyanate-derived amines in volunteers exposed to mixtures of TDI in exposure chambers showed absorption of both the 2,4- and 2,6-TDI isomers (Brorson et al. 1991; Skarping et al. 1991). These studies show that at least 20%, and possibly more, of an inhaled dose of TDI is absorbed, based on analysis of TDA levels in hydrolyzed urine. Brorson et al. (1991) exposed each of two men to a mixture of 70% 2,6-TDI with 30% 2,4-TDI in an exposure chamber at concentrations of 25, 50, and 70 μg/m^3 for 4-hour periods. Hydrolyzed plasma samples collected immediately after exposure showed detectable levels of 2,4-TDA after exposure to the highest concentration and detectable levels of 2,6-TDA after exposure to 50 and 70 μg/m^3. Analysis of 24-hour hydrolyzed urine samples showed excretion of 2,4-TDA estimated to represent 14–19% of the inhaled 2,4-TDI dose and levels of 2,6-TDA estimated to represent 17–23% of the inhaled 2,6-TDI dose. In another experiment, Skarping et al. (1991) exposed five men to a mixture of 52% 2,6-TDI and 48% 2,4-TDI at a concentration of 36 to 43 μg/m^3 for 7.5 hours, and measured TDA levels in hydrolyzed urine samples. Urinary levels of 2,4-TDA was estimated to represent 8–14% of the inhaled dose of 2,4-TDI and urinary levels of 2,6-TDA was estimated to represent 14–18% of the inhaled 2,6-TDI dose. As these urinary excretion levels reflected only the first 24–28 hours of excretion and fecal levels of TDI were not measured, the total absorption of 2,4- and 2,6-TDI may have been higher than estimated.

The results of a study in male F344 rats exposed to ^{14}C ring-labeled 2,4-TDI vapor (2 ppm) via inhalation for 4 hours suggest that approximately 61–90% of the radioactivity was absorbed; the remaining radioactivity was likely rapidly cleared from the respiratory tract and swallowed (Timchalk et al. 1994).

Using guinea pigs, Kennedy et al. (1989) showed a linear relationship between ^{14}C TDI exposure concentrations multiplied by exposure duration and radioactivity levels in blood samples taken immediately after 1-hour exposure to concentrations ranging from 0.0005 to 0.146 ppm, suggesting that absorption via the lung is not saturable in this concentration range. Blood samples taken during exposure via arterial cannula showed steady, essentially linear uptake during the 60-minute exposure period (Kennedy et al. 1989).

MDI. A single study examined the toxicokinetics of inhaled MDI in rats (Gledhill et al. 2005). The male Wistar rats were exposed, head only, to ^{14}C-4,4'-MDI (monomeric, as a condensation aerosol) at a

concentration of 2 mg/m^3 for 6 hours. A separate group of bile-cannulated rats was similarly exposed. Using data on urinary, biliary, and fecal excretion as well as radioactivity in the carcass measured 168 hours after exposure, the authors estimated that approximately 32% of the inhaled dose (calculated to be equivalent to 0.078 mg MDI per animal) was systemically available.

3.4.1.2 Oral Exposure

TDI. The absorption of TDI after oral exposure has only been examined in one gavage administration study. Timchalk et al. (1994) administered a single gavage dose of ^{14}C ring-labeled 2,4-TDI (60 mg/kg) to male F344 rats and analyzed excreta collected over the next 48 hours for radioactivity. Based on the measured radioactivity in the urine and carcass, at least 12% of the oral dose was absorbed; the investigators suggested that another 8% may have been eliminated through biliary excretion into the feces (Timchalk et al. 1994). It was also suggested that the radioactivity was absorbed as ^{14}C-2,4-TDA rather than as the parent compound. TDI absorption is likely to differ between ingestion and gavage administration due to differences in the pH of the oral cavity and stomach. Installation of TDI directly into the acidic stomach is likely to favor the formation of TDA, ureas, and polyureas. Comparatively, the neutral pH of the oral cavity would likely favor the binding of TDI to macromolecules and the formation of urea and polyureas.

MDI. No data on the absorption of MDI after oral exposure were located in the available literature.

3.4.1.3 Dermal Exposure

TDI. The limited available data demonstrate that TDI is absorbed across the skin, but the data are not adequate to estimate the rate and extent of absorption. Hoffman et al. (2010) detected <1% of a dermally-applied dose of 350 mg/kg body weight (12 mg/cm^2) ^{14}C-2,4-TDI in the urine, plasma, and carcasses of male rats exposed for 0.5, 1, or 8 hours; no detectable radioactivity was found in the feces. However, the animals were sacrificed immediately after exposure. Yeh et al. (2008) demonstrated dermal absorption of 2,4- and 2,6-TDI in male rats by measuring TDA levels in hydrolyzed urine for 6 days after a 5-hour dermal exposure to commercial-grade TDI at concentrations of 0.2, 1, and 5%. The maximum concentration in urine, as well as the area under the urinary concentration versus time curve both showed dose-related increases, providing evidence for dermal absorption.

MDI. Henriks-Eckerman et al. (2015) suggested that a comparison between urinary acetylated MDA levels at the end of the workshift to levels after a day off from work provides evidence of dermal

absorption of MDI since the workers wore respiratory protection. However, the investigators also noted that respiratory protection only reduced the inhalable amount by 60%.

When male rats were exposed to a topical dose of 15 or 165 mg/kg ^{14}C-4,4'-MDI and sacrificed at the end of the 8-hour exposure, or 24 or 120 hours after the commencement of exposure, <1% of the applied radioactivity was detected in the urine, feces, tissues, gut and its contents, and carcass (Hoffman et al. 2010). At both doses, the estimated amount of 4,4'-MDI absorbed was higher in rats sacrificed at later time points; for example, absorbed amounts were estimated to be 0.21, 0.66, and 0.88% of the applied dose of 165 mg/kg in rats sacrificed at 8, 24, and 120 hours after the beginning of exposure, respectively. However, in female rats exposed to topical doses of ~30 mg/kg ^{14}C-4,4'-MDI for 48 hours, 29–30% of the applied radioactivity was recovered in the feces during the first 48 hours after treatment, indicating significant dermal uptake (Vock and Lutz 1997). It is not clear whether the greater absorption suggested by the study by Vock and Lutz (1997) reflects a gender difference or an impact of longer exposure, or whether the rats in that study had unintended oral exposure via grooming. Hoffman et al. (2010) took measures to prevent oral exposure, while Vock and Lutz (1997) did not.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

TDI. Immediately after inhalation exposure to 2 ppm ^{14}C ring-labeled TDI for 4 hours, radioactivity levels were highest in the carcass, skin, gastrointestinal contents, and gastrointestinal tracts of male F344 rats (Timchalk et al. 1994). When examined 48 hours later, the highest percentage of recovered dose was found in the gastrointestinal contents (~17%), followed by the carcass (10%) and skin (6%). Table 3-9 shows the distribution of radioactivity immediately after exposure and 48 hours after exposure. Kennedy et al. (1994) measured radioactivity in tissues immediately after 4-hour exposure of rats to concentrations of 0.026, 0.143, and 0.821 ppm ^{14}C-2,4-TDI. The highest specific activities (μgEq/g) were located in the airways (trachea and lung) followed by the gastrointestinal tract (esophagus and stomach) and systemic circulation (blood, liver, kidney, spleen, and heart). When expressed as percent of dose /total tissue, the highest level was in the blood, followed by the liver or stomach, kidney or lung, and trachea. In guinea pigs exposed to ^{14}C-2,4-TDI concentrations ranging from 0.00005 to 0.146 ppm for 1, 4, or 5 hours, the highest levels of radioactivity were detected in the trachea and lung, followed by the kidney, heart, spleen, and liver (Kennedy et al. 1989).

Table 3-9. Tissue Distribution of ^{14}C in Male F344 Rats Exposed to ^{14}C Ring-Labelled 2,4-Toluene Diisocyanate Via Gavage or Inhalation

	Percent of administered dose after oral exposure (60 mg/kg)		Percent of recovered dose after inhalation exposure (2 ppm, 4 hours)	
Tissue	2 hours postdosing	48 hours postdosing	Immediately after exposure	48 hours postexposure
Blood	NA	0.05±0.02	NA	0.23±0.15
Gastrointestinal contents	65.82±8.35	2.56±0.84	9.76±2.31	16.63±9.18
Carcass	5.50±3.62	0.77±0.20	71.54±2.99	10.02±2.69
Gastrointestinal tract	10.10±3.09	0.10±0.05	3.75±1.56	0.76±0.37
Skin	1.12±0.53	0.15±0.02	9.86±3.12	5.59±1.61
Lung	0.99±0.52	<0.01	2.50±1.13	0.28±0.12
Liver	0.50±0.15	0.11±0.00	1.68±0.13	0.37±0.01
Kidney	0.08±0.02	0.02±0.00	0.69±0.08	0.25±0.04
Fat	<0.01	<0.01	0.02±0.00	<0.01
Total	83.18±7.19	3.77±0.87	–	34.14±11.53

Source: Timchalk et al. 1994

Kennedy et al. (1994) quantified the distribution of radioactivity in blood components after a 4-hour inhalation exposure of rats to ^{14}C-2,4-TDI. Radioactivity was primarily recovered from the plasma (74–87%), but radioactivity was also detected in the cell pellet. The plasma was fractionated by molecular weight, showing that the vast majority of the radioactivity (97–100%) was associated with high molecular weight (>10 kDa) components; electrophoresis was then used to demonstrate that the majority of the radioactivity was associated with a 70 kDa protein, which the authors suggested was likely albumin. Analysis of stomach contents by fractionation and electrophoresis showed that a higher proportion of the radioactivity in the stomach (28%) was in the low molecular weight fraction (<10 kDa) compared with the fraction in plasma. High performance liquid chromatography (HPLC) analysis of the low molecular weight fraction showed a TDA peak in addition to other products, demonstrating that TDA is not the primary reaction product after inhalation exposure. The authors postulated that the inhaled TDI reacted with macromolecules in the airway prior to being transported to the stomach, where proteolysis occurred, yielding the low molecular weight adducts.

Day et al. (1996) analyzed hemoglobin adducts of TDI in guinea pigs exposed to 1 ppm 2,4-TDI for 3 hours/day on 5 consecutive days, and identified several TDI-derived adducts that demonstrated that the isocyanate moiety was capable being transported from the lung into the blood and across the erythrocyte membrane to form a hemoglobin adduct.

MDI. Systemic distribution of radioactivity was measured in male Wistar rats exposed head-only for 6 hours to an aerosol of ^{14}C-4,4'-MDI (2 mg/m^3) (Gledhill et al. 2005). The results are shown in Table 3-10. As the table indicates, the largest percentages of received radioactivity were recovered from the respiratory and gastrointestinal tracts, but radioactivity was detected in all of the tissues examined (Gledhill et al. 2005). The authors suggested that the radioactivity in the gastrointestinal tract and its contents likely resulted from oral intake during grooming after the exposure period and/or mucociliary clearance of material from the respiratory tract (Gledhill et al. 2005).

3.4.2.2 Oral Exposure

TDI. In male F344 rats given a single gavage dose of 60 mg/kg ^{14}C ring-labeled TDI, the highest proportion of administered dose was recovered from the gastrointestinal tract and contents when sampled 2 or 48 hours postdosing (Timchalk et al. 1994). Radioactivity levels in the skin, lung, liver, and kidney reflected 1.1, 1.0, 0.5, and 0.08% of the administered dose, respectively, at 2 hours postdosing; lower concentrations were seen at 48 hours postdosing (Timchalk et al. 1994).

Table 3-10. Tissue Distribution of ^{14}C in Male Wistar Rats Exposed to ^{14}C Ring-Labelled 4,4'-Methylenediphenyl Diisocyanate Aerosol Via Inhalation

	Percent of received radioactivity		
Tissue	0 hours postexposure	24 hours postexposure	168 hours postexposure
Adrenals	0.025±0.01	0.021±0.005	0.025±0.004
Brain	0.051±0.018	0.031±0.007	<0.016±<0.006
Gastrointestinal	4.173±0.801	0.992±0.406	<0.141±<0.007
Gonads	0.356±0.022	0.201±0.032	0.054±0.021
Heart	0.375±0.068	0.157±0.041	0.053±0.013
Kidneys	0.524±0.089	0.363±0.03	0.103±0.014
Liver	3.379±0.756	2.004±0.408	0.424±0.058
Lungs	12.771±2.521	5.558±0.944	3.558±0.503
Nasal tissue (olfactory)	0.115±0.018	0.047±0.017	0.029±0.032
Nasal tissue (respiratory)	1.44±1.873	0.182±0.247	0.058±0.01
Esophagus	0.074±0.02	0.014±0.005	<0.039±<0.048
Pancreas	0.046±0.008	0.031±0.005	0.021±0.009
Spleen	0.102±0.021	0.071±0.012	0.043±0.009
Stomach	0.335±0.22	0.234±0.185	0.039±0.026
Thyroid	0.024±0.021	0.004±0.001	0.004±0.003
Trachea	0.167±0.168	0.095±0.103	0.012±0.002
Residual carcass	37.106±9.752	18.539±4.058	5.001±1.187
Total	61.063	54.901	5.159
Stomach contents	<0.921±<1.564	0.351±0.358	<0.103±<0.039
Gastrointestinal contents	31.787±5.133	13.177±5.487	0.617±0.13

Source: Gledhill et al. 2005

MDI. No data on the distribution of MDI after oral exposure were located in the available literature.

3.4.2.3 Dermal Exposure

TDI. The carcasses of male rats exposed to 330 mg/kg ^{14}C-2,4-TDI for 0.5, 1,0, or 8.0 hours via topical application contained 0.25, 0.44, and 0.52% of the applied radioactivity, respectively (Hoffman et al. 2010).

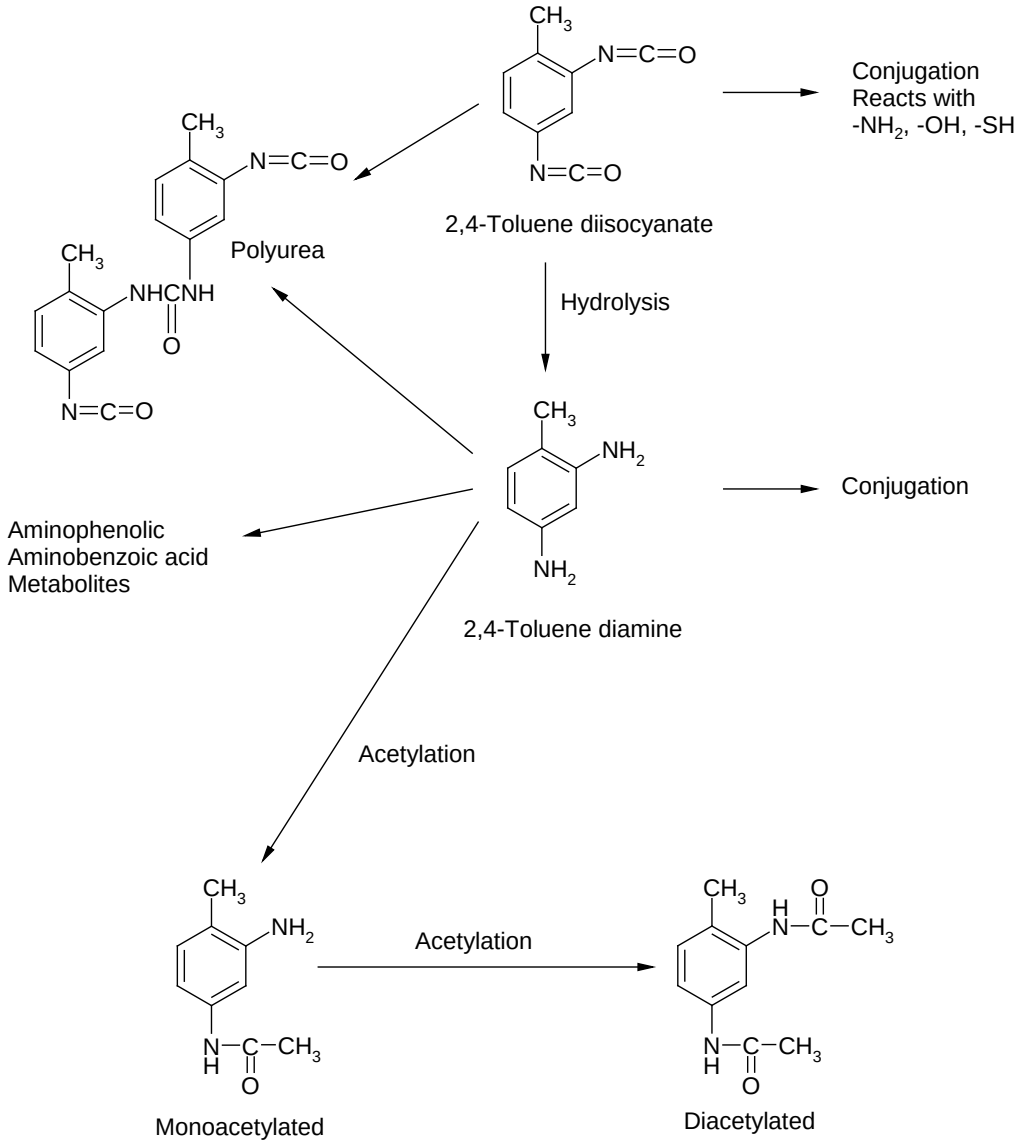
MDI. No radioactivity was detected in the tissues of male rats exposed for 8 hours to a topical dose of 15 or 165 mg/kg ^{14}C-4,4'-MDI and sacrificed 8, 24, or 120 hours after the commencement of exposure (Hoffman et al. 2010). Vock and Lutz (1997) detected small amounts of radioactivity (≤ 1% of applied radioactivity in total) in the lung, liver, kidney, and muscle of female rats exposed to topical doses of 11–15 mg/kg ^{14}C-4,4'-MDI for 24 hours or to 29–30 mg/kg for 48 hours. Of the applied radioactivity, 9–12% was recovered in the epidermis (Vock and Lutz 1997).

3.4.3 Metabolism

TDI reacts readily with sulfhydryl, amine, and hydroxyl groups, forming adducts with hemoglobin, glutathione, albumin, and other macromolecules. After gavage exposure, TDI is hydrolyzed in the gastrointestinal tract to TDA, which may be absorbed and metabolized further (acetylated, conjugated, or metabolized to aminophenolic or aminobenzoic acid compounds) (Timchalk et al. 1994). In the gut, TDA may also react with unhydrolyzed TDI to form polyurea polymers that pass unabsorbed through the gastrointestinal tract. In contrast, after inhalation exposure, little TDI, if any, is hydrolyzed to TDA; conjugation reactions are believed to represent be the primary fate of inhaled TDI. These route-specific differences in the fate of TDI were observed when rat urine was analyzed after gavage and inhalation exposure; after gavage exposure to TDI, 35% of the detected metabolites were free or acetylated TDA (the balance reflected acid-labile conjugates of TDI or TDA), while only 10% of the metabolites detected after inhalation exposure were acetylated TDA (Timchalk et al. 1994). The acidic pH of the stomach favors the hydrolysis of TDI to form TDA. At neutral pH levels, TDI is more likely to form polyurea polymers (as discussed in Sielken et al. 2012); thus, TDA formation may not occur following inhalation, ingestion, or dermal exposure to TDI.

Figure 3-4 shows the proposed metabolic scheme for 2,4-TDI in the rat.

Figure 3-4. Proposed Metabolic Scheme for 2,4-Toluene Diisocyanate in the Rat



Source: Timchalk et al. 1994

A single study evaluating the metabolism of MDI was located. In male rats exposed via inhalation to MDI aerosol, five metabolites were identified in the urine, feces, and bile (Gledhill et al. 2005). Table 3-11 shows the percentage of administered dose represented by each metabolite. Four metabolites were identified as N-acetylated and N-acetylated hydroxylated products of MDI, while the fifth could not be identified. The primary product detected in feces was proposed to be mixed molecular weight polyureas resulting from spontaneous reaction of MDI. No free MDA was detected in excreta or bile (Gledhill et al. 2005).

3.4.3.1 Inhalation Exposure

TDI. When rats were exposed by inhalation to 2 ppm 2,4-TDI for 4 hours, no free TDA was detected in the urine (Timchalk et al. 1994). A total of 0.26 μg equivalents of 2,4-TDA were detected in the hydrolyzed urine as mono- and diacetylated products, while 2.53 μg equivalents were detected as acid-labile conjugates of 2,4-TDI or TDA. Another inhalation study (Kennedy et al. 1994) showed that 95% of the TDI in plasma was conjugated to macromolecules, which the investigators suggested demonstrated that macromolecules successfully competed with hydrolysis to form the diamine.

In one study, TDI was shown to induce a decrease in CYP2B1 expression. Exposure of male Sprague-Dawley rats to commercial-grade TDI (80:20 mix of 2,4- and 2,6-TDI) at a concentration of 1 ppm for 8 hours resulted in decreased CYP2B1 mRNA (33%) and protein (40%) levels in the lung when compared with control rats (Pons et al. 2000). TDI exposure did not alter expression of other CYPs investigated (1A1, 2E1, or 3A1) or glutathione S-transferase (GST).

MDI. A total of five metabolites of 4,4'-MDI monomer were observed in the urine, feces, and bile of male rats exposed to 2 mg/m^3 radiolabeled MDI aerosol for 6 hours; four were identified by liquid chromatography-mass spectrometry (LC-MS) and LC-MS^3 analysis as N-acetylated and N-acetylated hydroxylated products of MDI, while the fifth could not be identified (Gledhill et al. 2005). The primary product detected in feces was proposed to be mixed molecular weight polyureas resulting from spontaneous reaction of MDI. Free MDA was not detected in excreta or bile. After acid hydrolysis of the 6-, 12-, and 24-hour urine samples, MDA was detected at concentrations of 483, 120, and 131 ng/mL, respectively. Analysis of the acid-hydrolyzed urine also revealed deacetylated products of the metabolites N,N'-diacetyl-4,4'-diaminobenzhydrol and N,N'-diacetyl 4,4'-diaminobenzophenone (Gledhill et al. 2005).

Table 3-11. Metabolites of Methylenediphenyl Diisocyanate (MDI) in Male F344 Rats Exposed to ^{14}C Ring-Labelled 4,4'-MDI Monomer Via Inhalation

	Percentage of administered dose in intact rats		Percentage of administered dose in bile-cannulated rats		
Metabolite	Urine	Feces	Urine	Bile	Feces
N,N'-Diacetyl-4,4'-diaminobenzhydrol	1	ND	6	1	ND
N,N'-Diacetyl-4,4'-diaminophenyl-methane	0.5	ND	4	4	ND
N-Acetyl-4, 4'-diaminophenylmethane	0.3	ND	ND	ND	ND
N,N'-Diacetyl 4,4'-diaminobenzophenone	0.4	ND	ND	ND	ND
Metabolite V; not identified	0.2	ND	<1	ND	ND
Proposed as mixed molecular weight polyureas derived from MDI	ND	56	ND	9	24
Total	2.4	56	10	14	24

ND = not detected

Source: Gledhill et al. 2005

3.4.3.2 Oral Exposure

TDI. After rats were given a single gavage dose of 60 mg/kg 2,4-TDI, 2,4-TDA was detected (by HPLC) in the urine collected during the first 12 hours postdosing (Timchalk et al. 1994). In the urine, 2.08 µg equivalents of free 2,4-TDA were detected, while monoacetylated, and diacetylated 2,4-TDA were measured to be 5.12 and 8.17 µg equivalents of 2,4-TDA. Approximately 44.51 µg equivalents existed in the urine as acid-labile conjugates of 2,4-TDA and/or 2,4-TDI (Timchalk et al. 1994). The relevance of these gavage data in which the TDI is instilled into the acidic stomach to human ingestion is questionable. At neutral pH levels, such as found in the mouth, TDI is more likely to react with other TDI molecules to form polyurea polymers than to hydrolyze to TDA (as discussed in Sielken et al. 2012).

MDI. No data on the metabolism of MDI after oral exposure were located in the available literature.

3.4.3.3 Dermal Exposure

TDI. No data on the metabolism of TDI after dermal exposure of humans or animals were located in the available literature.

MDI. No data on the metabolism of MDI after dermal exposure of humans or animals were located in the available literature.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

TDI. Budnik et al. (2011) evaluated urinary excretion of diamines following specific inhalation challenge exposure to known concentrations ranging from 0.5 to 30 ppb of 2,4-TDI (n=18) or 2,6-TDI (n=18). The subjects were workers with prior exposure to these compounds who were being evaluated for occupational asthma. Levels of TDA in the spot urine samples collected over the following 24 hours were subjected to acid hydrolysis prior to analysis by gas chromatography (GC)/MS. In subjects exposed to 2,4- and 2,6-TDI, creatinine-corrected urinary levels of the corresponding diamines peaked at 4.1 and 4.8 hours, respectively. The half-life for TDA in urine was estimated to be 6 hours. Subjects exposed to higher concentrations of either isomer of TDI (mean exposure 1,569 ppb) did not exhibit correspondingly higher urinary peak levels of TDA when compared with the low exposure group (496 ppb).

The plasma elimination rate for both 2,4- and 2,6-TDI was estimated to average 21 days in workers chronically exposed to airborne concentrations between 0.4 and 4 $\mu g/m^3$ TDI (mixture of 2,4- and 2,6-TDI with varying composition) (Lind et al. 1996).

TDA levels in hydrolyzed urine in humans experimentally exposed for 4–7.5 hours to mixtures of 2,4- and 2,6-TDI exhibited a biphasic pattern, with an initial rapid phase followed by a slower phase (Brorson et al. 1991; Skarping et al. 1991). The half-time for urinary excretion in the initial rapid phase was estimated to be between 1.6 and 2.5 hours for 2,6-TDI and between 1.9 and 5 hours for 2,4-TDI (Brorson et al. 1991; Skarping et al. 1991). The half-time for the slower phase was reportedly about 5 hours for both isomers (Skarping et al. 1991).

In guinea pigs, 2,3-TDI is cleared slowly from the blood. Kennedy et al. (1989) observed a gradual decline in blood radioactivity over the course of 72 hours following exposure of guinea pigs to concentrations of ^{14}C-2,4-TDI ranging from 0.004 to 0.336 ppm. Radioactivity remaining in the blood at 72 hours postexposure persisted at that level for a second week, suggesting that the molecule to which the radioactivity was bound was saturated, and that the adduct did not have a rapid turnover rate (Kennedy et al. 1989).

The primary excretory pathway for 2,4-TDI in rats exposed via inhalation was fecal (Timchalk et al. 1994). Forty-eight hours after a 4-hour exposure to 2 ppm ^{14}C ring-labeled 2,4-TDI, male F344 rats excreted 47% of the recovered radioactivity in the feces and 15% in the urine. No radioactivity was detected in exhaled CO_2 or volatile organics (Timchalk et al. 1994). Detection of significant amounts of radioactivity in the gastrointestinal contents both immediately after exposure (10% of recovered dose) and 48 hours later (17% of recovered dose) (Timchalk et al. 1994) suggests biliary excretion of 2,4-TDI.

MDI. In addition to evaluating urinary excretion of diamines in workers undergoing specific inhalation challenge with TDI, Budnik et al. (2011) measured levels of 4,4'-MDA in acid hydrolyzed urine following specific inhalation challenge exposure to 4,4'-MDI (0.5–30 ppb; n=36 subjects). The peak level of 4,4'-MDA in urine occurred at 14 hours after exposure. Urinary excretion of 4,4'-MDA was slower and more prolonged than that of the TDAs, and excretion was not complete during the 24-hour study period. In addition, the excretion time course was longer in those subjects exposed to higher concentrations of 4,4'-MDI (mean exposure 1,569 ppb) compared with those exposed to lower concentrations (mean exposure 496 ppb).

Male rats exposed (head only) to aerosols of ^{14}C-4,4'-MDI monomer for 6 hours at 2 mg/m^3 excreted the majority of the received radioactivity in the feces (80%), with about 5% excreted in urine during the 168-hour follow-up time (Gledhill et al. 2005). In bile duct-cannulated rats exposed similarly, biliary excretion was estimated to be 14% of the dose and urinary excretion was 12%.

3.4.4.2 Oral Exposure

TDI. After gavage exposure to a 60 mg/kg ^{14}C ring-labeled 2,4-TDI, 81% of the administered dose was recovered in the feces and 8% was recovered in the urine of male F344 rats; total radioactivity recovered represented 94% of the administered dose (Timchalk et al. 1994). Quantifiable levels of radioactivity were not detected in exhaled CO_2 or volatile organics.

MDI. No data on the elimination of MDI after oral exposure were located in the available literature.

3.4.4.3 Dermal Exposure

TDI. Hoffman et al. (2010) detected <1% of a dermally-applied dose of 330 mg/kg ^{14}C-TDI in the urine of rats after exposure durations up to 8 hours; no radioactivity was detected in the feces. Yeh et al. (2008) evaluated the kinetics of urinary excretion of 2,4- and 2,6-TDI in male rats by measuring urinary TDA for 6 days after topical application of commercial-grade TDI (80:20 mixture of 2,4- and 2.6-TDI) at concentrations of 0.2, 1, and 5%. 2,4- and 2,6-TDA were measured in acid-hydrolyzed urine samples collected at 12-hour intervals. The results are shown in Table 3-12. For both compounds and regardless of applied concentration, the maximum concentration in urine was reached during the first 12-hour interval. At the highest exposure level, urinary excretion was not complete at the end of the 6-day collection period, but was essentially complete at the lower concentrations. The half-life for urinary elimination of 2,4- and 2.6-TDA ranged between 18.4 and 26.6 hours. The data readily fit a first-order kinetic linear model ($p<0.05$), but the pattern at the highest exposure demonstrated a non-linear saturation at 60 hours after Cmax was reached (Yeh et al. 2008).

MDI. Hoffman et al. (2010) detected only small amounts of radioactivity in the feces of male rats exposed via dermal application of 15 or 165 mg/kg ^{14}C-4,4'-MDI for 8 hours and sacrificed 8, 24, or 120 hours after treatment. In contrast, approximately 29–30% of the applied radioactivity was recovered in the feces during a 48-hour exposure of female rats to topical doses of ~30 mg/kg ^{14}C-4,4'-MDI (Vock

Table 3-12. Kinetics of Urinary Toluene Diamine (TDA) Excretion in Rats Exposed to Toluene Diisocyanate Via Topical Application

	2,4-TDA			2,6-TDA		
Applied dose	0.2%	1%	5%	0.2%	1%	5%
Tmax (hours)	12	12	12	12	12	12
Cmax (µg/mL)	0.062± 0.009	0.238± 0.060	6.116± 0.429	0.056± 0.004	0.268± 0.060	3.777± 0.384
AUC (µg*hour/mL)	2.186± 0.376	8.395± 0.919	158.599± 5.517	2.046± 0.263	10.558± 0.538	133.994± 20.35
Accumulative amount (µg)	2.682± 0.631	12.940± 4.224	83.843± 29.542	2.622± 0.779	14.978± 2.628	69.810± 11.541
k (1/hour)	0.0376± 0.002	0.0341± 0.003	0.0325± 0.003	0.0329± 0.0020	0.0339± 0.0027	0.0264± 0.004
t1/2 (hours)	18.4±0.8	20.4±01.5	21.5±2.2	21.1±1.3	20.5±1.6	26.6±3.7

Source: Yeh et al. 2008

and Lutz 1997). Hoffman et al. (2010) took measures to prevent oral exposure of the rats via grooming, while Vock and Lutz (1997) did not. In both studies, recovery of radioactivity in the urine was <1% of the applied dose (Hoffman et al. 2010; Vock and Lutz 1997).

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

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

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

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

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

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

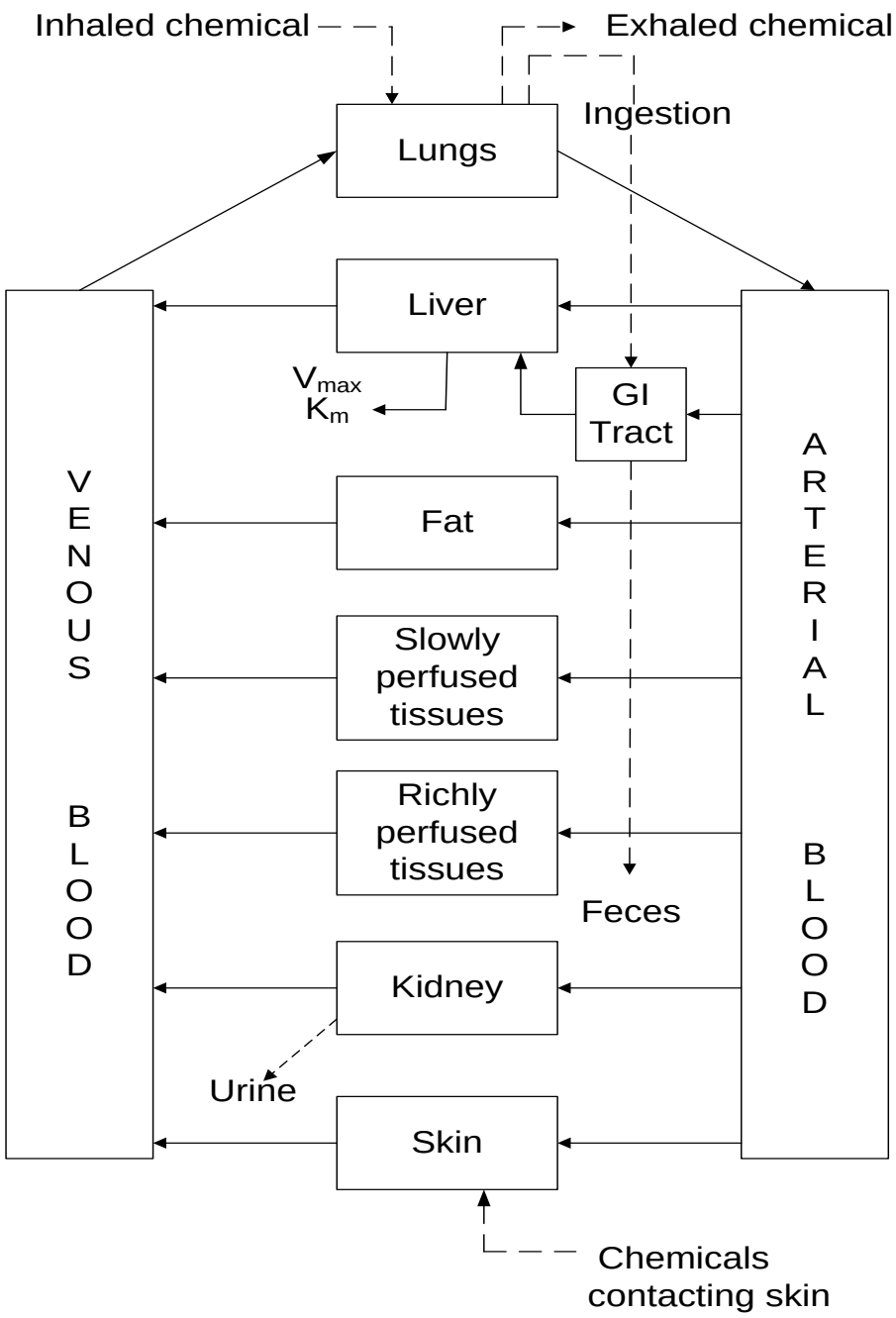
No physiologically based pharmacokinetics models for TDI or MDI were located in the available literature.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

The metabolic fate of TDI is route-dependent. After oral exposure, TDI is hydrolyzed in the gastrointestinal tract to TDA, which may be absorbed and metabolized further (acetylated, conjugated, or metabolized to aminophenolic or aminobenzoic acid compounds) (Timchalk et al. 1994). In the gut, TDA may also react with unhydrolyzed TDI to form polyurea polymers that pass unabsorbed through the gastrointestinal tract. In contrast, after inhalation exposure, little TDI, if any, is hydrolyzed to TDA; conjugation reactions are believed to represent be the primary fate of inhaled TDI. As a consequence of these route differences, exposure to TDI via the gastrointestinal tract will likely result in higher tissue concentrations of TDA and its downstream products than would occur after inhalation exposure.

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



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.

Source: Krishnan and Andersen 1994

3.5.2 Mechanisms of Toxicity

The respiratory tract is the primary target of TDI and MDI toxicity resulting in declines in lung function and occupational asthma; chronic airway inflammation likely plays a key role in both effects. The mechanisms of diisocyanate-induced occupational asthma have been more extensively investigated than those involved in reduced lung function. The pathogenesis of TDI/MDI asthma has not been fully elucidated; it appears to be multifaceted and involves a number immunological and non-immunological mechanisms. TDI/MDI occupational asthma has many similar features to allergic asthma, including persistent airway inflammation and subsequent airway hyperresponsiveness; however, there are several features present in TDI/MDI asthma not seen in allergic asthma including airway neutrophilia, increases in interleukin (IL)-8 levels, low prevalence of diisocyanate-specific IgE antibodies, and lack of association with atopy (Furusho et al. 2006). It is believed that diisocyanates bind to airway cell proteins and are taken up by epithelial cells resulting in cytokine and chemokine production and cellular recruitment, which leads to airway inflammation (Kim et al. 2010). Wisnewski et al. (2013) speculated that albumin is the primary protein target of diisocyanate reactivity; the diisocyanate-albumin conjugate can trigger innate and adaptive cellular responses associated with airway inflammation and asthma. Glutathione serves as a carbamoylating intermediate through which diisocyanate is transported from the airways to the blood where there are higher levels of albumin. This shuttle mechanism could explain the rapid accumulation of diisocyanate-albumin conjugates in the peripheral blood in animals exposed to diisocyanates (Wisnewski et al. 2013).

The immunological mechanisms appear to involve hypersensitivity response, although other types of immune response are likely involved. There is suggestive evidence that all types of hypersensitivity are involved. An immediate response to a diisocyanate challenge is likely indicative of Type 1 hypersensitivity, which is mediated by IgE. Specific IgE antibodies to TDI-HSA (Baur and Fruhmann 1981; Cvitanovic e t al. 1989; Park and Nahm 1996; Park et al. 1999; Pezzini et al. 1984; Sharifi et al. 2013) or MDI-HSA (Hur et al. 2008; Pezzini et al. 1984; Tse et al. 1985) have been found. However, only a small percentage of TDI and MDI workers with occupational asthma have elevated levels of IgE, suggesting that other mechanisms are likely involved. The delayed response to a TDI or MDI challenge is suggestive of one or more subtype of Type IV hypersensitivity. This type of response is typically driven by leukotrienes, chemokines, and cytokines synthesized by activated mast cells and CD4+ Th2 cells. A study of CD4 knockout mice sensitized to TDI showed a significant reduction in airway hyperresponsiveness to a TDI challenge as compared to wild-type controls. A marked reduction of pulmonary inflammation by neutrophils, lymphocytes, eosinophils, and macrophage infiltration and

decreases in Th2 cytokines—IL-4, IL-5, and IL-13—were also observed in the CD4 knockout mice (Matheson et al. 2005). The role of CD4+ Th2 subtype of Type IV hypersensitivity in diisocyanate-induced asthma is supported by the findings of increased IL-4 and IL-6 levels in the bronchoalveolar lavage (BAL) fluid of rats sensitized to TDI (Zheng et al. 2001a, 2001b). Increases in the production of IL-1β, IL-1α, and tumor necrosis factor (TNF)-α expression were observed in the lungs of mice with TDI-induced asthma; increases in IL-1β and TNF-α expression were also observed in the lung biopsy samples from workers with TDI-induced asthma (Johnson et al. 2005). Studies in TDI-sensitized mice in which IL-1β or IL-1α was suppressed showed that they have unique and overlapping roles (Johnson et al. 2005). A central role for TNF-α in the propagation of airway inflammation and hyperresponsiveness is supported by a study of TNF-α deficient mice that found a reduction in TDI-induced inflammation, airway hyperresponsiveness, and migration of airway dendritic cells to the draining lymph nodes (Matheson et al. 2002). The increases in IFN-γ and TNF-α observed in TDI sensitized mice also support a Th1 response mechanism (Świerczyńska-Machura et al. 2014). There is also some evidence to support mechanisms for the other two subtypes of Type IV hypersensitivity. A significant reduction in the Th1 cytokine, interferon-γ (INF-γ), was observed in CD4 knockout mice (Matheson et al. 2005). In contrast, Zheng et al. (2001a) did not find significant differences in Th1 cytokines (IL-2 or IFN-γ) levels in TDI-sensitized rats, as compared to controls. The Matheson et al. (2005) study also provides some evidence of the CD8+ subtype of Type IV hypersensitivity; reductions in airway hyperresponsiveness and pulmonary inflammation were observed in CD8 knockout mice sensitized to TDI as compared to TDI-sensitized wild-type mice.

In addition to immune hypersensitivity mechanism, there is evidence to suggest that other immune and non-immune mechanisms are involved in TDI/MDI-induced inflammation and airway hyperresponsiveness. *In vitro* studies in bronchial epithelial cells have showed enhanced production of IL-8 in the presence of TDI-HSA conjugate (Lee et al. 2003; Ogawa et al. 2006). The IL-8 attracts and activates neutrophils, and increased neutrophil counts have been observed in the BAL fluid of sensitized workers exhibiting a delayed response to a TDI challenge (Fabbri et al. 1987). A marked infiltration of eosinophils, as well as neutrophils, was observed in the central and peripheral airways of TDI-sensitized rats (Zheng et al. 2001a); in sensitized mice, increased leukocyte levels were observed in the BAL fluid with the eosinophils having the greatest increase (Zheng et al. 2004). De Vooght et al. (2013) showed that granulocytes played a key role in TDI-induced airway hyperresponsiveness. Ogawa et al. (2006) showed that TDI-HSA increased cytokine and chemokine production through the epidermal growth factor (EGFR) and p38 mitogen-activated protein kinase (MAPK) pathways. Results from studies conducted by

Pham et al. (2014) suggest that TDI can bind to tissue transglutaminase and that this conjugate can induce specific IgG antibody production, which can increase airway inflammation.

Intercellular adhesion molecule-1 (ICAM-1) plays a key regulatory role in TDI-induced inflammation by mediating the adhesion of blood leukocytes to the vascular epithelium (Furusho et al. 2006). In ICAM-1 knockout mice sensitized and challenged with TDI, there is a reduction in neutrophil, lymphocyte, eosinophil, and macrophage airway infiltration, a blocking of airway hyperresponsiveness, and marked decreases in TNF-α, IL-4, IL-5, and IFN-γ levels in the BAL fluid. Another mediator of airway inflammation that is overexpressed in response to TDI-HSA conjugates is vascular endothelial growth factor (VEGF) (Zhao et al. 2009). Overexpression of VEGF can result in increased vascular permeability and Th2 cell sensitization. Incubating bronchial cells with TDI-HSA resulted in increased cell permeability; however, neutralizing VEGF partially inhibited this increase in cell permeability (Zhao et al. 2009). Kim et al. (2011) found higher VEGF levels in workers with TDI-induced asthma, as compared to asymptomatic TDI-exposed workers.

Several investigators have shown that oxidative stress plays an essential role in diisocyanate-induced inflammation. Studies of workers with TDI- or MDI-induced asthma have shown increased transferrin levels and decreased ferritin levels (Hur et al. 2009; Kim et al. 2010). Ferritin is used for detoxification during oxidative stress-induced inflammation. Kim et al. (2010) found that TDI suppressed the synthesis of ferritin light chain in human airway epithelial cells. Several other antioxidant proteins were also found to be downregulated by TDI, including heme oxygenase-1, thioredoxins-1, glutathione peroxidase, peroxiredoxin-1, and catalase. Heme oxygenase-1/ferritin light chain expression was likely suppressed through the MAPK-Nrf2 signaling pathway (Kim et al. 2010). Studies in epithelial cells have also shown that TDI exposure induces the generation of reactive oxygen species (Hur et al. 2009).

3.5.3 Animal-to-Human Extrapolations

Kennedy et al. (1994) compared the quantities of TDI-derived components (as radioactivity) in the blood of guinea pigs, rats, and humans exposed by inhalation to ^{14}C- 2,4-TDI in several studies, and observed a linear relationship between the log-transformed microgram equivalents of the tolyl group per mL of blood and the log-transformed exposure concentration x time metric (ppm-hours). This observation suggests limited interspecies differences in the absorption of inhaled TDI into the blood stream. No data on interspecies differences in the pharmacokinetic behavior of MDI were located in the available literature.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No studies were located regarding endocrine disruption in humans and/or animals after exposure to TDI or MDI. No *in vitro* studies were located regarding endocrine disruption of TDI or MDI.

3.7 CHILDREN'S SUSCEPTIBILITY

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

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

Children sometimes differ from adults in their susceptibility to adverse health effects from exposure to hazardous chemicals, but whether there is a difference depends on the chemical(s) (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to exposure-related health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life that are most sensitive to disruption from exposure to hazardous substances. Damage from exposure in one stage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). Past literature has often described the fetus/infant as having an immature (developing) blood-brain barrier that is leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-CNS interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the brain, there are differences between fetuses/infants and adults that are toxicologically important. These differences mainly involve variations in physiological transport

systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; whether these mechanisms provide protection for the developing brain or render it more vulnerable to toxic injury is an important toxicological question. Chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns given their low glomerular filtration rate and not having developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

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

Limited data for TDI and MDI on children's susceptibility were identified. In the absence of data, it is assumed that the respiratory tract would be the most sensitive target of toxicity for both compounds. Two studies have examined the developmental toxicity and both reported skeletal effects. Exposure to TDI on GDs 6–15 resulted in poorly ossified cervical vertebrae at a concentration also resulting in markedly reduced maternal weight gain and respiratory symptoms (Tyl et al. 1999a). An increase in the occurrence of asymmetric sternebrae was observed in rats; maternal toxicity was limited to a decrease in food consumption (Buchsmann et al. 1996).

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to TDI and MDI are discussed in Section 3.8.1.

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

adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by TDI and MDI are discussed in Section 3.8.2.

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

3.8.1 Biomarkers Used to Identify or Quantify Exposure to TDI and MDI

A number of potential urinary and plasma biomarkers of exposure to TDI and MDI have been investigated. TDA, likely released by hydrolysis of protein adducts, has been measured in plasma and in acid- or alkaline-hydrolyzed urine as a biomarker of exposure to 2,4- and 2,6-TDI (Austin et al. 2007; Brorson et al. 1991; Geens et al. 2012; Sennbro et al. 2004; Sepai et al. 1995; Skarping et al. 1991; Tinnerberg et al. 1997, 2014). Both Geens et al. (2012) and Sennbro et al. (2004) observed strong correlations (coefficients ranging from 0.75 to 0.88) between personal air concentrations of 2,4- and 2,6-TDI and plasma and urinary levels of 2,4-, 2,6-, and total TDA in occupationally exposed persons. Similarly, the diamine metabolite of MDI (MDA) has been studied as a biomarker of exposure (Sabbioni et al. 2007; Schutze et al. 1995; Sennbro et al. 2003, 2006; Sepai et al. 1995). Sennbro et al. (2006) reported statistically significant, but not strong, correlation coefficients of 0.51–0.65 for the association between personal air measurements of MDI and plasma or urinary levels of MDA (samples collected the same day as the air measurements; urinary samples were hydrolyzed). The authors noted that there was significant interindividual variation. Tinnerberg et al. (2014) also found correlations between TDA levels in hydrolyzed urine (creatinine adjusted or specific gravity adjusted) and TDA levels in hydrolyzed plasma; strong correlations were also found for MDA levels in hydrolyzed urine and hydrolyzed plasma. It was noted that GSTM1 polymorphisms modified the association between urine and plasma TDA levels.

To facilitate the distinction between background levels of exposure and occupational exposure, Sennbro et al. (2005) measured plasma and hydrolyzed urinary 2,4- and 2,6-TDA and MDA in workers with and without occupational exposure to isocyanates. Upper reference limits on the background biomarker levels were calculated using the receiver operator characteristic curve method; the results are shown in Table 3-13. TDA was detected infrequently in unexposed persons (detection frequencies ranging from

2 to 15%), while MDA was detected in nearly all (97%) urinary and plasma samples (Sennbro et al. 2005).

Diamines may be present in the plasma and urine as a result of exposure to the corresponding diisocyanate or exposure to the diamine itself; thus, this biomarker is not specific to isocyanate exposure. Sabbioni et al. (2010, 2012) and Kumar et al. (2009) developed methods for measuring TDI and MDI adducts of albumin that are specific to isocyanates. Sabbioni et al. (2012) detected 2,4- and 2,6-TDI adducts with the lysine of albumin in blood samples taken from 10 workers 26 days after they were accidentally exposed to TDI (details of the exposure were not provided). Three lysine adducts were detected: N^{ε}-[({3-amino-4-methylphenyl}amino)carbonyl]-lysine (3A4MP-Lys); N^{ε}-[({5-amino-2-methylphenyl}amino)carbonyl]-lysine (5A2MP-Lys); and N^{ε}-[({3-amino-2-methylphenyl}amino)-carbonyl]-lysine (3A2MP-Lys). The adducts were detected at concentrations ranging from 29 to 269 fmol/mg. Repeat analysis of selected samples showed coefficients of variation ranging from 2.1 to 6.6% for the three adducts. Half-lives of the albumin adduct levels were estimated to be 21.7 days for 3A4MP-Lys, 40.3 days for 5A2MP-Lys, and 19.6 days for 3A2MP-Lys.

Sabbioni et al. (2010, 2016) likewise detected albumin adducts of MDI in workers exposed to MDI; the adducts were identified as N^{6}-[({4-[4-aminobenzyl]phenyl}amino)carbonyl]lysine (MDI-Lys) and N^{6}-[({4-[4-acetylaminobenzyl]phenyl}amino)carbonyl]lysine (AcMDI-Lys). The level of MDI-Lys was correlated with MDA in acid- and alkaline-hydrolyzed urine, but not with measurements of hemoglobin adducts of MDA. The authors (Sabbioni et al. 2010) noted that measurement of hemoglobin adducts only would have underestimated the number of exposed workers; only 27% of workers exhibited hemoglobin adducts of MDI, while albumin adducts were observed in 64% of workers.

In summary, recent exposure to diisocyanates may be reflected in TDA or MDA levels in acid- or alkaline-hydrolyzed urine or plasma, but these biomarkers may also be present in urine and plasma as a result of exposure to the diamines themselves (TDA and MDA). Background levels of TDA and MDA in urine and plasma should be considered in the interpretation of measured values from subjects with

Table 3-13. Upper Reference Limits for Biomarkers of Exposure to Toluene Diisocyanate and Methylenediphenyl Diisocyanate

Biomarker	Median[a] (µg/L)	Range[a] (µg/L)	Frequency of detection[a] (%)	Upper reference limit (µg/L)	Sensitivity (%)	Specificity (%)
Urinary 2,4-TDA	<0.1	<0.1–0.4	7	0.4	94	100
Plasma 2,4-TDA	<0.1	<0.1–0.1	2	0.1	100	100
Urinary 2,6-TDA	<0.1	<0.1–0.2	15	0.2	97	100
Plasma 2,6-TDA	<0.1	<0.1–0.1	2	0.2	99	100
Urinary MDA	0.2	<0.05–3.0	97	0.5	100	97
Plasma MDA	0.2	<0.05–0.4	97	0.4	88	100

[a]TDA and MDA measured in 120 unexposed workers from five workplaces in Sweden.

MDA = methylenediphenyl amine; TDA = toluene diamine

Source: Sennbro et al. 2005

unknown exposure. Finally, serum levels of albumin adducts of TDI or MDI are specific to diisocyanate exposure and, due to their longer half-life, may be useful in assessing exposure over the preceding weeks. These biomarkers have been shown to be useful for identifying exposure to TDI or MDI; however, no biomarkers have been identified that allow for quantification of exposure.

3.8.2 Biomarkers Used to Characterize Effects Caused by TDI and MDI

One of the prominent health effects associated with inhalation exposure to TDI or MDI is the induction of occupational asthma. Several tests have been developed to diagnosis occupational asthma; these include measurement of peak expiratory flow rate, nonspecific bronchial provocation testing, specific immunological testing, skin-prick testing, specific inhalational challenge testing, and nasal lavage testing (Jolly et al. 2015; Ott et al. 2007). With the exception of the specific immunological and specific inhalational challenge tests, these tests are not specific to TDI or MDI exposure. Although specific inhalational challenge testing is considered one of the better tests for diagnosing sensitizer-induced occupational asthma (Jolly et al. 2015; Ott et al. 2007; Vandenplas et al. 2014), the American College of Occupational and Environmental Medicine notes that it is a highly technical test and has the potential for inducing severe adverse effects, including fatalities (Jolly et al. 2015). Several investigators have evaluated the usefulness of specific immunological tests, TDI-/MDI-specific IgE and IgG levels, for diagnosing TDI-/MDI-induced occupational asthma. As discussed in Section 3.2.1.2, a number of occupational exposure studies have reported IgG- or IgE-specific antibodies to TDI-HSA in workers with TDI-induced asthma (Baur and Fruhmann 1981; Cvitanovic et al. 1989; Park and Nahm 1996; Park et al. 1999; Pezzini et al. 1984; Sharifi et al. 2013) or MDI-HSA in MDI workers (Hur et al. 2008; Pezzini et al. 1984; Tse et al. 1985; Zeiss et al. 1980). However, specific IgG or IgE antibodies were typically observed in a small percentage of TDI workers (16–57%). In a small study of MDI workers (Budnik et al. 2013), MDI-specific IgE antibodies were detected in four of seven workers with confirmed MDI-induced asthma, none of the four workers with hypersensitivity pneumonitis, and none of the six asymptomatic workers. In contrast, IgG antibody levels were detected in four of seven workers with asthma, four of four subjects with hypersensitivity pneumonitis, and one of six asymptomatic workers. In a review conducted by Wisnewski (2007), isocyanate-specific serum IgE has been found in up to 50% of workers. It is noted that isocyanate-specific IgE levels have a half-life of approximately 2 days and levels can drop below the detection limit following brief periods with no exposure (Wisnewski 2007). Palikhe et al. (2011) also noted that the prevalence of IgG antibodies was not a reliable biomarker because the prevalence was too low. This is less of an issue for IgG, which has a half-life of approximately 30 days. The American College of Occupational and Environmental Medicine concluded that there is insufficient

evidence to assess the usefulness of IgE testing for low molecular weight antigens (Jolly et al. 2015); it is noted that this recommendation is not specific to isocyanates.

Several studies have examined other biomarkers that could be used for early diagnosis of TDI-induced asthma. Significantly lower matrix metalloproteinase-9 (MMP-9) level and higher VEGF levels were found in workers with TDI-induced asthma, as compared to asymptomatic workers (Kim et al. 2011; Palikhe et al. 2011). The sensitivity and specificity of the MMP-9 were 79.7 and 80.0%, respectively (Kim et al. 2011). Combining several variables (MMP-9, VEGF, and interleukin-8) increased the sensitivity to 82.6%, but decreased the specificity to 75.8%. Kim et al. (2012) found that the levels of vitamin D-binding protein (VDBP) were significantly higher in workers with isocyanate-induced occupational asthma, as compared to asymptomatic workers from the same working environment, or in unexposed healthy subjects; the sensitivity and specificity was 69 and 81%, respectively. Ye et al. (2006) examined the usefulness of three cytokeratins (CK8, CK18, and CK19) for identifying TDI-induced asthma. Significantly higher IgG antibody levels of CK8, CK18, and CK19 were found in the workers with TDI-induced asthma as compared to asymptomatic workers, subjects with allergic asthma, and healthy subjects. The sensitivity and specificity for C8, CK18, and CK19 antibodies were 18.2 and 95.2%, 26.2 and 93.5%, and 26.2 and 93.5%, respectively.

3.9 INTERACTIONS WITH OTHER CHEMICALS

No studies were identified examining the influence of other chemical on the toxicity or toxicokinetics of TDI or MDI.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

There are data to suggest that there is a genetic susceptibility factor that may predispose certain individuals to develop occupational asthma as a result of exposure to TDI or MDI. Several investigators

have examined possible associations between genetic polymorphisms and diisocyanate-induced asthma. Vucesoy et al. (2012) demonstrated that genetic variants of antioxidant defense genes are associated with increased susceptibility to diisocyanate (TDI, MDI, or HDI)-induced asthma in a study of diisocyanate workers with confirmed occupational asthma, workers reporting respiratory symptoms who did not react to diisocyanate challenge, and asymptomatic HDI workers. Significant associations between diisocyanate-induced asthma and three types of variant genotypes (manganese superoxide dismutase [SOD2] rs4880, microsomal epoxide hydrolase [EPHX1] 2740171, and a glutathione S-transferase [GSTP1] rs1695) were noted. Blindow et al. (2015) also found greater responses to specific inhalation challenges among symptomatic isocyanate workers with GST1 deletions and a higher risk of developing IgE-mediated reactions in workers with GSTM1 deletions. In a study of 84 workers with TDI-induced occupational asthma, higher bronchial hyperresponsiveness was observed in TDI workers with asthma with polymorphisms of catenin alpha 3, alpha-T-catenin (CTNNA3) (Kim et al. 2009). Similar results were observed in a second study of diisocyanate workers; increased risks of CTNNA3 polymorphisms were found among workers with occupational asthma, but not among workers without asthma (Bernstein et al. 2013).

Several studies have examined the frequency of human leukocyte antigen class II (HLA) haplotypes among with TDI-induced asthma. Higher frequencies of haplotypes DRB1*15-DPB1*05 (Kim et al. 2006) and DRB1*1501-DQB1*0602-DPB1*0501 (Choi et al. 2009) and the allele DQB1*0503 and the allelic combination of DQB1*0201/0301 (Bignon et al. 1994) were found among workers with TDI-induced asthma. Similarly, Yucesoy et al. (2014) found increases in the susceptibility to diisocyanate-induced asthma among diisocyanate workers with single nucleotide polymorphisms in HLA-E HLA-DPB1, HLA-DOA, or HLA-DQA2 genes. Both Kim et al. (2006) and Beghe et al. (2004) found an alteration in the distribution of HLA class I antigens in subjects with TDI-induced asthma. Ye et al. (2010) found no differences in the allelic, genotypic, or haplotypic frequencies of beta 2-adrenergic receptor gene (ADRB2) polymorphisms among TDI workers with occupational asthma, asymptomatic workers, or controls with no TDI exposure. However, significant associations between two ADRB2 polymorphisms (Arg16Gly and Arg173Arg single nucleotide polymorphisms) and the prevalence of specific IgE antibodies to TDI-HSA were found among TDI workers and a significantly higher TDI-HSA specific IgE sensitization was found in workers with the ADRB2 ht1/ht1 homozygote. Broberg et al. (2008) found that an increased risk of eye symptoms was associated with the CYP1A1*2A variant and an increased risk of wheezing was associated with CYP1A1*2B. Studies by Yucesoy et al. (2015, 2016) identified several gene and single nucleotide polymorphisms that may be associated with susceptibility to diisocyanate-induced asthma. Single nucleotide polymorphisms mapping to several genes including

TNFα, TGB1, PTGS1, PTGS2, HERC2, CDH17, and ODZ3 have been found to contribute to diisocyanate-induced asthma susceptibility.

Studies examining clinical features of subjects with suspected occupational asthma found no differences in the incidence of atopy among workers who reacted to a TDI challenge and workers not reacting to the TDI challenge (Mapp et al. 1988; Moscato et al. 1991; Paggiaro et al. 1984). Significantly fewer subjects reacting to TDI were found to be current smokers; although a higher percentage of ex-smokers were found among the TDI reactors (Moscato et al. 1991; Paggiaro et al. 1984). One study found a higher number of workers with positive skin tests to common allergens among the reactors (Paggiaro et al. 1984).

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to TDI and MDI. 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 TDI and MDI. When specific exposures have occurred, poison control centers, board certified medical toxicologists, board-certified occupational medicine physicians and/or other medical specialists with expertise and experience treating patients overexposed to TDI and MDI can be consulted for medical advice. The following texts provide specific information about treatment following exposures to TDI and MDI:

Blanc PD. 2018. Section II: Specific poisons and drugs: Diagnosis and treatment: Isocyanates. In: Poisoning & drug overdose. 7th ed. McGraw-Hill Education. https://accessmedicine.mhmedical.com/book.aspx?bookid=2284. May 30, 2018.

Leikin JB, Paloucek FP. 2008. Methylene diisocyanate and toluene diisocyanate. In: Poisoning and toxicology handbook. 4th ed. Boca Raton, FL: CRC Press, 824; 857-858.

Vena J, McKay C. 2007. Isocyanates and related compounds. In: Haddad and Winchester's clinical management of poisoning and drug overdose. 4th ed. Philadelphia, PA: Saunders Elsevier, 1317-1322.

Additional relevant information can be found in the front section of this profile under QUICK REFERENCE FOR HEALTH CARE PROVIDERS.

3.11.1 Reducing Peak Absorption Following Exposure

No studies were identified that examined reducing peak absorption of TDI or MDI following exposure.

3.11.2 Reducing Body Burden

No studies were identified that examined reducing body burden of TDI or MDI following exposure.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Several studies have examined the effectiveness of asthma medication or corticosteroid medication inhibiting the asthmatic reaction and nonspecific airway reactivity associated with TDI exposure in sensitized individuals. An acute treatment course with ketotifen, atropine, slow-release verapamil, or cromolyn did not prevent dual and/or late asthmatic reactions in TDI-sensitized individuals receiving an inhalation challenge with TDI (Mapp et al. 1987; Paggiaro et al. 1987; Tossin et al. 1989). Ketotifen, verapamil, and cromolyn also did not alter bronchial responsiveness to methacholine (Mapp et al. 1987; Tossin et al. 1989). In contrast, administration of beclomethasone or prednisone prevented the asthmatic reaction and airway hyperresponsiveness following a TDI inhalation challenge (Boschetto et al. 1987; Mapp et al. 1987). Slow-release theophylline partially inhibited the immediate and late asthmatic reaction to TDI but did not alter airway hyperresponsiveness (Mapp et al. 1987). In subjects receiving a 5-month treatment with beclomethasone, there was an improvement in the response to TDI inhalation challenge 1 month post-treatment; however, a similar improvement was found in untreated controls (Maestrelli et al. 1993). However, beclomethasone treatment did improve airway hyperresponsiveness to methacholine, a finding not observed in the untreated controls.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of TDI and MDI 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 adverse health effects (and techniques for developing methods to determine such health effects) of TDI and MDI.

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 risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

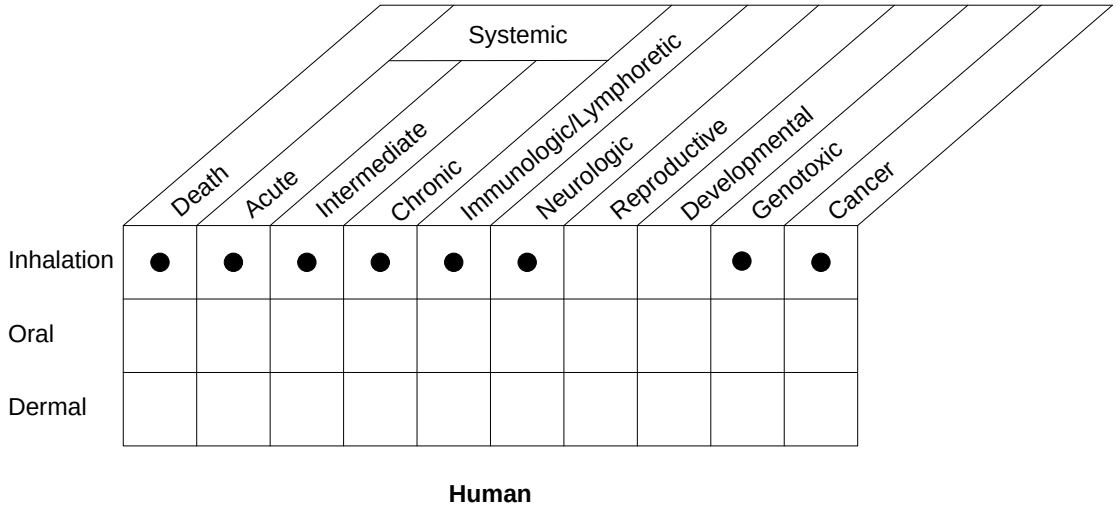
3.12.1 Existing Information on Health Effects of TDI and MDI

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to TDI and MDI are summarized in Figure 3-6 and 3-7, respectively. The purpose of these figures is to illustrate the existing information concerning the health effects of TDI and MDI. 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.

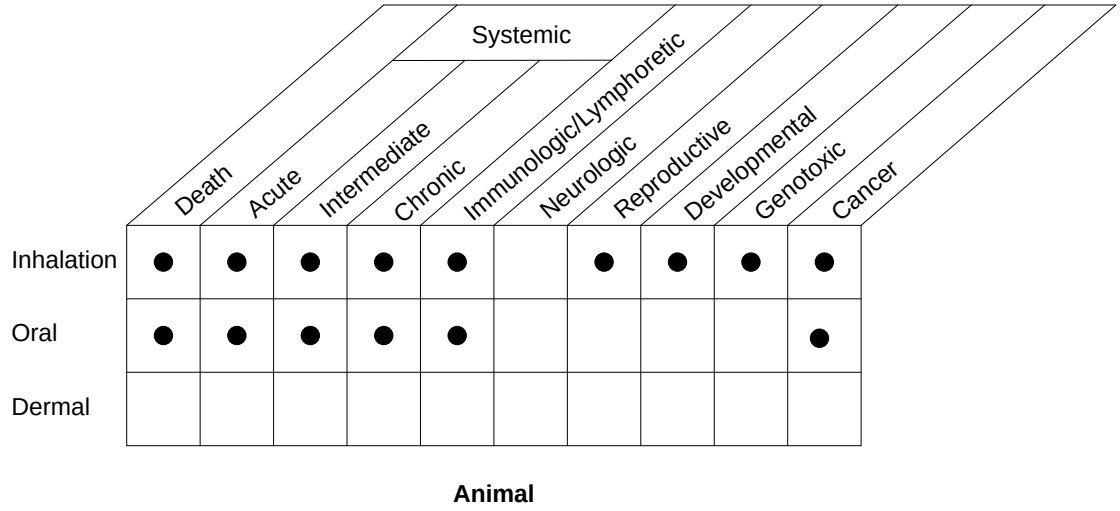
3.12.2 Identification of Data Needs

Acute-Duration Exposure. Although several case reports of single exposures to TDI (Axford et al. 1976; Le Quesne et al. 1976; Schmidt-Nowara et al. 1973; Singer and Scott 1987; Vandenplas et al. 1992; Yoshizawa et al. 1989) and MDI (Chang and Karol 1984; Suojalehto et al. 2011) have reported respiratory effects following an acute exposure, they did not include monitoring data. Several acute exposure experimental studies have examined lung function following a single exposure to TDI (Chester et al. 1979; Vandenplas et al. 1999). Animal studies have examined the toxicity of TDI (Aoyama et al. 1994; Arts et al. 2008; Buckley et al. 1984; Gagnaire et al. 1996; Gordon et al. 1985; Johnson et al. 2007; Marek et al. 1999; Sangha and Alarie 1979; Wong et al. 1985; Zissu 1995) and MDI (Marek et al. 1999); the observed effects on the respiratory system include histological damage to the nasal cavity and lungs and increased airway responsiveness. In general, these studies did not examine end points outside of the target tissues, the respiratory tract. The database for TDI was considered adequate for derivation of an acute-duration inhalation MRL; however, a repeated exposure study examining lung function in humans would provide support for this MRL. The database was not considered adequate for derivation of an acute-duration inhalation MRL for MDI and studies are needed that provide concentration-response data. Acute-duration data on the toxicity of TDI following oral exposure are limited to single and 14-day exposure studies that found increases in mortality and decreases in body weight gain (NTP 1986); other

Figure 3-6. Existing Information on Health Effects of Toluene Diisocyanate



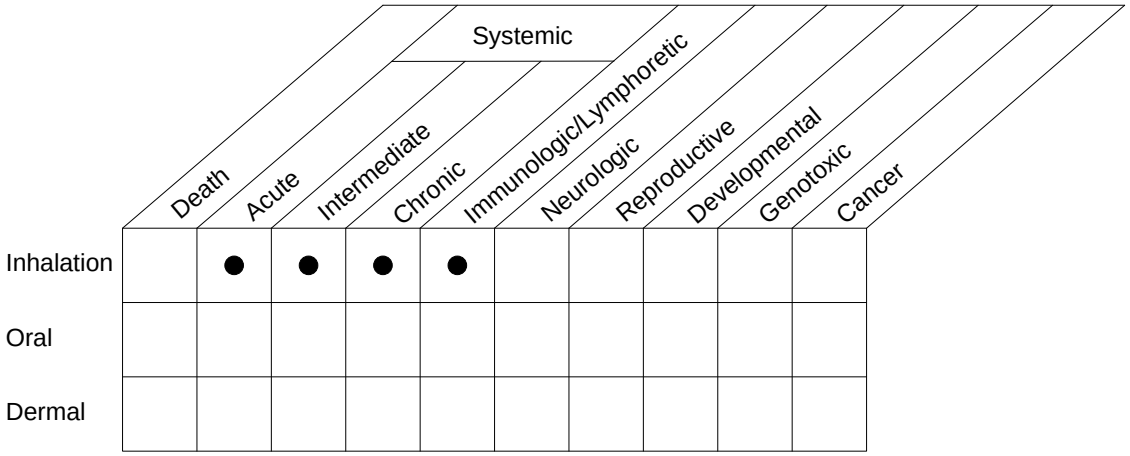
Human



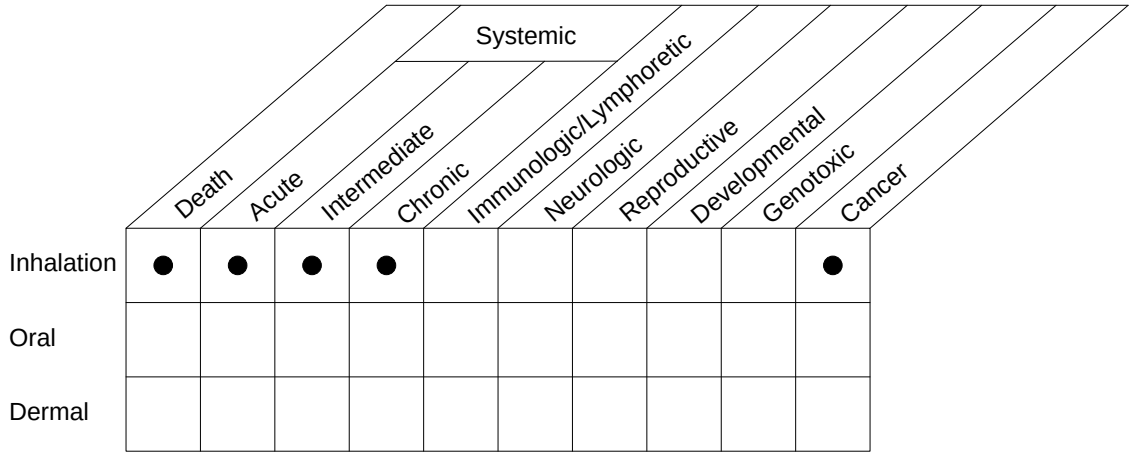
Animal

● Existing Studies

Figure 3-7. Existing Information on Health Effects of Methylenediphenyl Diisocyanate



Human



Animal

● Existing Studies

end points were not examined. No studies examined the acute-duration oral toxicity of MDI. Studies that examine the dermal toxicity of TDI and MDI are needed, particularly since ocular and dermal irritation has been reported in workers exposed to airborne TDI and MDI.

Intermediate-Duration Exposure. Occupational exposure studies typically involve chronic-duration exposure; however, there is suggestive evidence that effects can occur after several months of exposure (Clark et al. 1998). Three studies have examined the toxicity of TDI to the respiratory tract of animals following intermediate-duration exposure (Matheson et al. 2005; Wong et al. 1985; Zissu 1995). The studies reported histological damage in the nasal cavity and lungs and an increase in airway hyperresponsiveness. One study examined the intermediate-duration toxicity of MDI in animals (Marek et al. 1999), but the study was limited to the examination of airway hyperresponsiveness and did not include a histological examination of the respiratory tract. Although the database was considered inadequate for the derivation of intermediate-duration inhalation MRLs, the chronic-duration inhalation MRLs could be used for intermediate duration.

Chronic-Duration Exposure and Cancer. The chronic toxicity of TDI and MDI has been extensively investigated in occupational studies of production facilities and polyurethane manufacturing facilities and in workers applying polyurethane varnishes (Bodner et al. 2001; Burge 1982; Clark et al. 1998, 2003; Diem et al. 1982; Jang et al. 2000; Liss et al. 1988; Mapp et al. 1998; Moscato et al. 1991; Musk et al. 1982; Ott et al. 2000; Paggiaro et al. 1986, 1993; Sulotto et al. 1990; Zammit-Tabona et al. 1983). These studies provide strong evidence that the respiratory tract is the most sensitive target of TDI or MDI toxicity. Longitudinal studies of TDI workers provide sufficient monitoring data to allow for the derivation of a chronic-duration inhalation MRL (Clark et al. 1998; Diem et al. 1982). Monitoring data in the MDI studies were not considered adequate. The chronic toxicity of TDI and MDI has also been investigated in two animal studies (Loeser 1983; Reuzel et al. 1994); these studies also identify the respiratory tract as the most sensitive target. A chronic-duration rat study (Reuzel et al. 1994) was used to derive a chronic-duration inhalation MRL for MDI. Data on the chronic toxicity of orally administered TDI was investigated by NTP (1986). No long-term studies on the dermal toxicity of TDI or MDI were located.

There are limited human data on the carcinogenicity of TDI or MDI. Three studies of polyurethane foam manufacturing workers provide some suggestive evidence of an increased lung cancer risk, but the association with diisocyanates was not established (Mikoczy et al. 2004; Schnorr et al. 1996; Sorahan and Nichols 2002). No significant increases in lung cancer were observed in animals following TDI (Loeser

1983) or MDI (Reuzel et al. 1994) exposure. Increases in tumor incidence were observed in a chronic-duration gavage study of TDI (NTP 1986). The relevance of the findings in this gavage study to humans exposed to TDI via ingestion has been questioned due to likely toxicokinetic differences between ingestion and gavage administration of this very reactive compound. Additional studies are needed to address these concerns.

Genotoxicity. Both TDI and MDI have been tested for genotoxicity in prokaryotic and mammalian systems *in vitro*. TDI is not stable in most *in vitro* test systems; TDA is formed rapidly in the vehicles used in available genotoxicity tests, and it has been suggested that TDA is responsible for positive mutagenicity tests of TDI (Seel et al. 1999). Mixed results have been found in *in vivo* tests for genotoxic end points. However, the interpretation of the results of some of the studies is limited by methodological problems or poor reporting. Additional *in vivo* studies would facilitate assessing the interpretation of the genotoxicity data.

MDI also degrades to MDA in *in vitro* test systems using DMSO or EGDE, but at a much slower rate than TDI does. When MDI was tested for genotoxicity in EGDE (in which MDI is more stable), the results were negative, while positive results were seen when DMSO was used as the solvent (Herbold et al. 1998). There is little information on genotoxicity of MDI in humans or non-human mammalian systems tested *in vivo*.

Reproductive Toxicity. The available data on the reproductive toxicity of TDI consists of a 2-generation study in rats exposed via inhalation (Tyl et al. 1999b) in which no effects on reproductive parameters were observed. No data on the reproductive toxicity of MDI in humans or animals exposed by any route were located in the available literature.

Developmental Toxicity. A single developmental toxicity study of TDI in rats exposed via inhalation (Tyl et al. 1999a) showed poorly ossified cervical centra at an exposure concentration that also resulted in maternal toxicity; no other exposure-related effects were seen in the offspring. Similarly, there is one study of MDI developmental toxicity in rats exposed via inhalation (Buchsmann et al. 1996); an increased incidence of litters with asymmetric sternebrae was the only treatment-related effect. There are no data on the developmental toxicity of TDI or MDI via oral or dermal exposure routes.

Immunotoxicity. Available literature did not include human or animal studies evaluating immunological effects after exposure to TDI or MDI. Occupational asthma observed in TDI (Mapp et al.

1988; Moller et al. 1986; Moscato et al. 1991; Padoan et al. 2003; Paggiaro et al. 1984, 1986; Saetta et al. 1995) and MDI (Bonauto et al. 2005; Burge 1982; Chang and Karol 1983; Hur et al. 2008; Liss et al. 1988; Suojalehto et al. 2011; Woellner et al. 1997; Zammit-Tabona et al. 1983) workers may be the result of immunotoxicity; however, additional research is needed to identify the mechanism of toxicity.

Neurotoxicity. The database for diisocyanates (including both TDI and MDI) does not include any information on neurological effects of chronic-duration exposure. Human and/or animal studies are warranted given the suggestive evidence for long-term impairment after acute exposure (Le Quesne et al. 1976; Singer and Scott 1987). In addition, no information on potential neurotoxicity of MDI was located; animal and/or mechanistic studies are needed to evaluate this end point.

Epidemiological and Human Dosimetry Studies. Numerous studies have examined the toxicity of TDI (Bodner et al. 2001; Clark et al. 1998, 2003; Diem et al. 1982; Mapp et al. 1998; Moscato et al. 1991; Ott et al. 2000; Paggiaro et al. 1986, 1993) and MDI (Burge 1982; Jang et al. 2000; Liss et al. 1988; Musk et al. 1982; Sulotto et al. 1990; Zammit-Tabona et al. 1983) in occupationally exposed subjects; additionally, two studies have examined possible adverse health outcomes in residents living near TDI sources (Nuorteva et al. 1987; Wilder et al. 2011). These studies provide strong evidence that the respiratory tract is the most sensitive target resulting in occupational asthma, respiratory symptoms, and impaired lung function. However, many studies are lacking reliable monitoring data, particularly in studies examining workers exposed to MDI. Several occupational exposure studies have also assessed the potential association between inhalation exposure to diisocyanates and cancer (Mikoczy et al. 2004; Schnorr et al. 1996; Sorahan and Nichols 2002) and found suggestive evidence between work in the polyurethane foam manufacturing industry and lung cancer in female workers, but an association with diisocyanate exposure was not established. Significant limitations of all three studies included the lack of control for confounding factors, such as smoking and alcohol consumption, and coexposure to mixtures of compounds including those other than diisocyanates. Continued epidemiological research focused on improving exposure estimates (for example, using biomarkers of exposure) and control for confounding is recommended.

Biomarkers of Exposure and Effect.

Exposure. Biomarkers of exposure to TDI and MDI include the diamine hydrolysis products (TDA and MDA) as well as hemoglobin and albumin adducts of the isocyanates. Improvements in the standardization of methods used to pretreat biological samples (e.g., acid- and alkaline-hydrolysis) prior

to analysis could help to refine the predictive relationship between levels of metabolites or adducts in the samples and exposure.

Effect. Given the continued decline in lung function and the delay in recovery when TDI- or MDI-sensitized workers remain in jobs involving TDI/MDI exposure (Banks et al. 1990; Mapp et al. 1988; Padoan et al. 2003; Paggiaro et al. 1984; Park and Nahm 1997), biomarkers that would allow for early detection of sensitization are needed. Investigators have identified several potential biomarkers of effect including MMP-9 (Kim et al. 2011), VEGF (Kim et al. 2011), cytokeratins (Ye et al. 2006), which may be useful for early detection of occupational asthma. Additional studies in sensitized workers are needed to evaluate the usefulness of these biomarkers and others for early detection of TDI and/or MDI sensitization.

Absorption, Distribution, Metabolism, and Excretion. Human and animal data suggest that TDI and MDI are absorbed to some extent via all exposure routes. Both TDI and MDI combine readily with biological macromolecules including hemoglobin, albumin, and others. As a consequence of their reactivity, these compounds or their reaction products are often found at higher concentrations at the site of entry into the body early in exposure, and may continue to be distributed from the site of entry long after exposure has terminated. Once in the body, conjugated TDI and MDI are distributed to a large number of tissues, albeit at low levels.

The metabolic fate of TDI depends on the exposure route. After oral exposure, TDI is hydrolyzed in the gastrointestinal tract to TDA, and subsequently either absorbed and metabolized further or reacted with unhydrolyzed TDI to form polyurea polymers that pass unabsorbed through the gastrointestinal tract. However, after inhalation exposure, the primary fate of TDI appears to be conjugation reactions; little TDI, if any, is hydrolyzed to TDA. In humans exposed experimentally, urinary excretion of the TDI metabolite TDA exhibits a biphasic pattern, with an initial rapid phase followed by a slower phase. The primary route of TDI elimination after inhalation or oral exposure of rats is via the feces, which may include material absorbed and excreted via the bile.

Few data on the metabolism and elimination of MDI were identified in the available literature. Like TDI, MDI is excreted primarily in the feces of rats after inhalation exposure, and there is evidence for biliary excretion of MDI. As there are no data on the pharmacokinetic behavior of MDI after oral exposure in humans or animals, research on the route-dependence of MDI metabolism would be particularly beneficial.

PBPK models of TDI and MDI pharmacokinetics have not yet been developed.

Comparative Toxicokinetics. There are few data on species differences in the toxicokinetics of TDI, and no data on this issue for MDI. Research to assess species differences in MDI toxicokinetics would provide important information regarding the extrapolation from animal toxicity information to human effects.

Methods for Reducing Toxic Effects. Several investigators have examined the effectiveness of asthma medication or corticosteroids for treating occupational asthma induced by TDI (Boschetto et al. 1987; Maestrelli et al. 1993; Mapp et al. 1987; Paggiaro et al. 1987; Tossin et al. 1989). Although some beneficial effects were observed when asthmatic subjects were challenged with TDI during the treatment course (Boschetto et al. 1987; Mapp et al. 1987), long-term benefits have not been found (Maestrelli et al. 1993). Since a large number of subjects with occupational asthma do not recover even after exposure cessation, additional research is needed on the treatment of TDI- or MDI-induced occupational asthma. In addition, studies are needed to assess the treatment of other TDI- or MDI-related health effects such as decreased lung function.

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

No information on children's susceptibility to TDI or MDI toxicity was identified and it is not known if children would be more susceptible to the irritating properties of TDI or MDI. Although TDI/MDI exposure primarily occurs in the workplace, communities living near TDI or MDI sources or the commercial use of products containing uncured TDI or MDI can result in exposure to children. Two studies have examined communities living near a TDI source (Nuorteva et al. 1987; Wilder et al. 2011), one of these studies included children (Nuorteva et al. 1987); however, the data were not analyzed by age group. Given the potential for exposure, studies are needed to address this data gap.

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

3.12.3 Ongoing Studies

The following ongoing studies pertaining to TDI and MDI have been identified in the National Institutes of Health (NIH) Research Portfolio Online Reporting Tools (RePORTER 2016) database.

Adam Wisnewski at L2 Diagnostics, LLC is developing two immunoassays that can be used to biomonitor MDI exposure in the workplace. The two biomarkers being investigated are MDI-specific IgG antibodies and MDI albumin conjugates