CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of NDMA. 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. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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

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 by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to NDMA, but may not be inclusive of the entire body of literature.

Animal inhalation studies are presented in Table 2-1 and Figure 2-2, and animal oral studies are presented in Table 2-2 and Figure 2-3; no dermal data were identified for NDMA.

Levels of significant exposure (LSEs) 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 (SLOAELs) 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

endpoint 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 endpoints. 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. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of NDMA are indicated in Tables 2-1 and 2-2 and Figures 2-2 and 2-3.

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

Studies examining the health effects of NDMA after inhalation, oral, or dermal exposure and discussed in this profile include 24 human studies and 89 animal studies. Most of the human studies examined associations between dietary intake of NDMA and various cancers. As shown in Figure 2-1, the vast preponderance of the available data consists of studies of animals exposed by oral administration (at doses ranging from 0.0007 to 50 mg/kg/day) in which hepatic effects, cancer, and/or survival were assessed. Very few data are available for other endpoints. A substantial number of studies were identified in which NDMA was administered via intraperitoneal (i.p.) injection in rats as an animal model of liver fibrosis or cirrhosis. However, these studies do not contribute to the understanding of NDMA health effects or dose-response relationships and are thus not discussed in this profile.

- **Hepatic Effects:** Data on the hepatic effects of NDMA are largely limited to studies of animals exposed by oral administration. In these studies, NDMA induced severe liver injury (hemorrhagic necrosis, fibrosis, and/or cirrhosis) in a wide range of species (rats, mice, hamsters, monkeys, dogs, cats, guinea pigs, and mink) after all exposure durations. Human data on the hepatic effects of NDMA are limited to case reports.

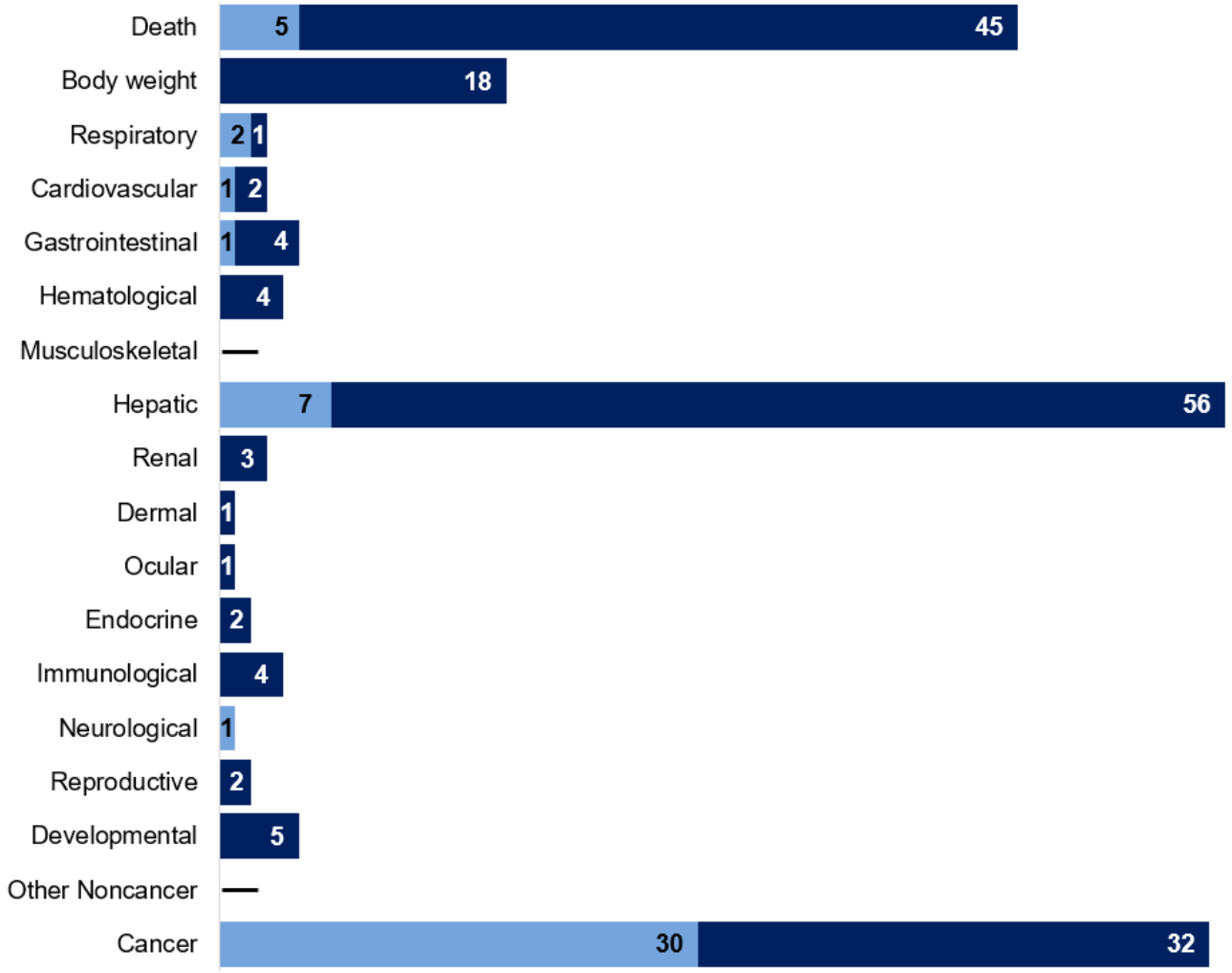
- **Developmental Effects:** Very limited data pertaining to developmental effects of NDMA were located, but the available studies suggest that oral exposure may result in fetal or neonatal mortality after acute- or intermediate-duration exposure in animals. The available information on potential teratogenic effects of NDMA is insufficient, as the only studies examining this endpoint were limited by lack of controls, lack of maternal toxicity data, and/or uncertain treatment schedule.

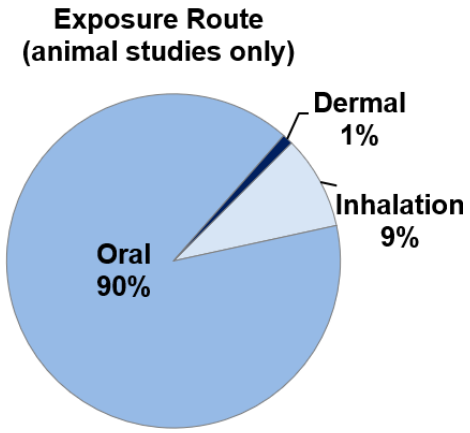
- **Cancer Effects:** In a study of occupational exposure by inhalation, cumulative NDMA exposure was associated with higher risks of gastric, liver, bladder, and prostate cancers, and also with increased risks of leukemia and multiple myeloma. Epidemiological studies have reported associations between NDMA exposure in the diet and gastric and colorectal cancers. In animals exposed by inhalation, NDMA has induced liver, lung, and kidney tumors in rats and mice, and nasal tumors in rats. Oral exposure to NDMA primarily induces liver and lung tumors in rats and mice and has also induced kidney tumors in these species and testicular tumors in rats. Increased incidences of liver tumors were also observed in hamsters and mink after oral exposure to NDMA.

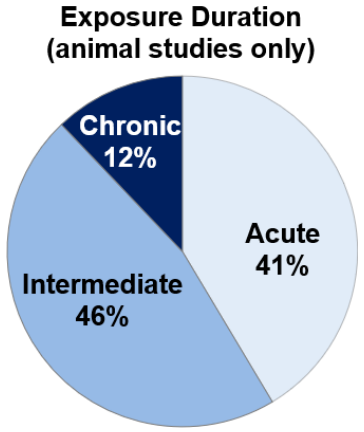
Figure 2-1. Overview of the Number of Studies Examining N-Nitrosodimethylamine (NDMA) Health Effects*

Most studies examined the potential carcinogenicity, hepatic effects, and lethality of NDMA

Fewer studies evaluated health effects in humans than animals (counts represent studies examining endpoint)







*Includes studies discussed in Chapter 2. A total of 113 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to N-Nitrosodimethylamine – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
1	Rat (NS) 10 M	4 hours, once	41–188	CS,GN	Death			78	LC_{50}
					Hepatic			78	Hemorrhagic necrosis
Jacobson et al. 1955									
2	Mouse (NS) 10 F	4 hours, once	39–67	CS,GN	Death			57	LC_{50}
					Hepatic			57	Hemorrhagic necrosis
Jacobson et al. 1955									
3	Dog (beagle) 3 M	4 hours, once	16–144	BC, CS, OF GN, HP, HE	Death			16	2/3 died; all died at higher exposures
					Hepatic			16	Hemorrhagic necrosis
Jacobson et al. 1955									
CHRONIC EXPOSURE									
4	Rat (BD) 6–12 NR	Lifetime, 2 days/week, 0.5 hours/day	50, 100	HP	Cancer			50	CEL: nasal tumors
Druckrey et al. 1967									
5	Rat (Sprague-Dawley) 36 F	At least 52 weeks, 5 days/week, 4 hours/day	0, 0.04, 0.2, 1	LE, BW, HP	Death			1	Reduced survival (median survival 9 months less than controls)
					Bd wt	0.2		1	>20% decrease in body weight at the end of exposure
					Cancer			0.04	CEL: nasal tumors
Klein et al. 1989, 1991									
6	Rat (Wistar) 36–51 M, F	25 months, continuous	0, 0.002, 0.07	HP	Cancer			0.07	CEL: liver, lung, kidney tumors
Moiseev and Benemanski 1975									

Table 2-1. Levels of Significant Exposure to N-Nitrosodimethylamine – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
7	Mouse (BALB/c) 30–68 M, F	17 months, continuous	0, 0.002, 0.07	HP	Cancer	0.002		0.07	CEL: liver, lung, kidney tumors
Moiseev and Benemanski 1975									

BC = blood chemistry; Bd wt or BW = body weight; CEL = cancer effect level; CS = clinical signs; F = female(s); GN = gross necropsy; HE = hematology; HP = histopathology; LC_{50} = concentration producing 50% death; LE = lethality; LOAEL = lowest-observed-adverse-effect level; m = male(s); NOAEL = no-observed-adverse-effect level; NR = not reported; OF = organ function

Figure 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Inhalation
Acute (≤14 days)

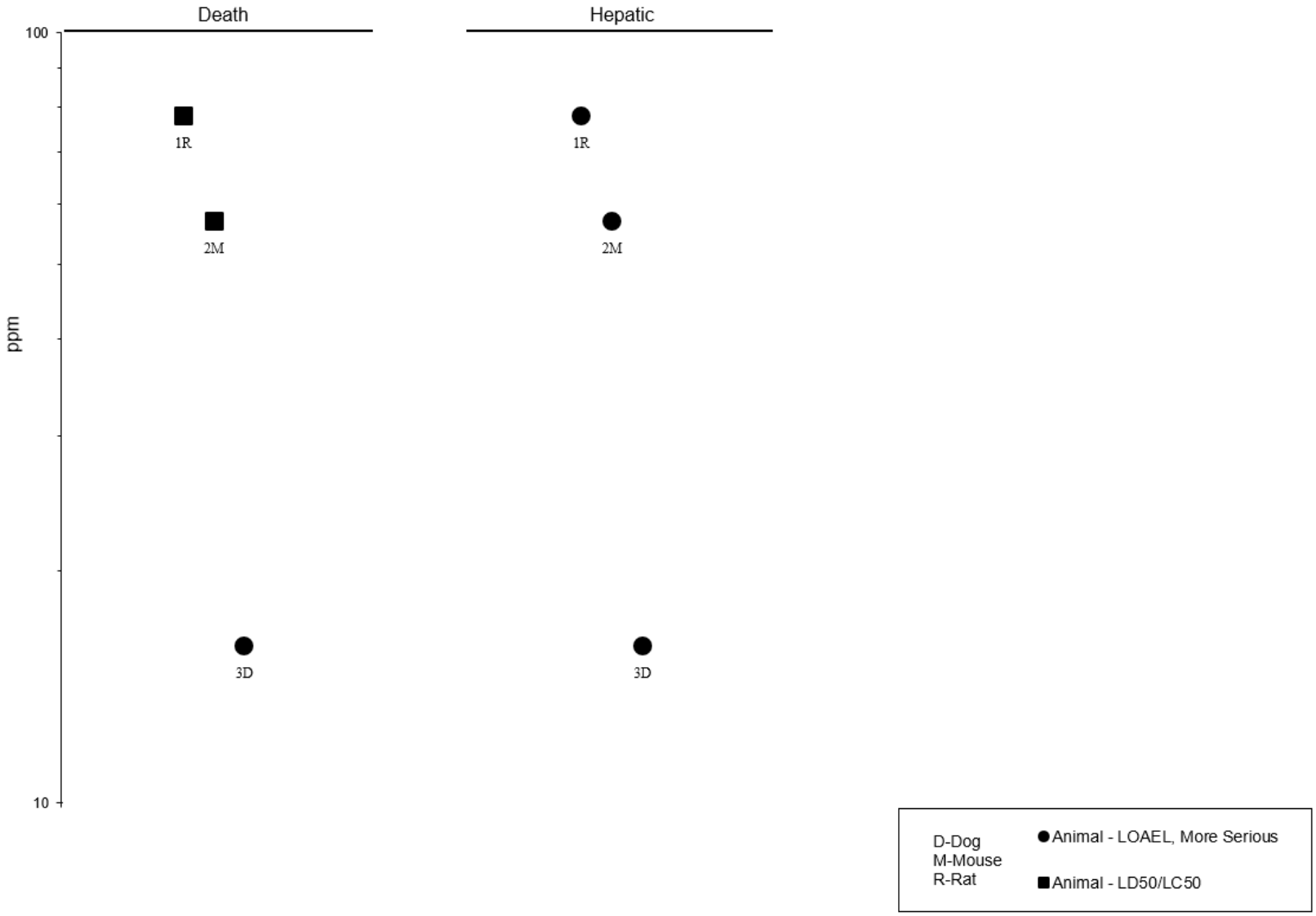
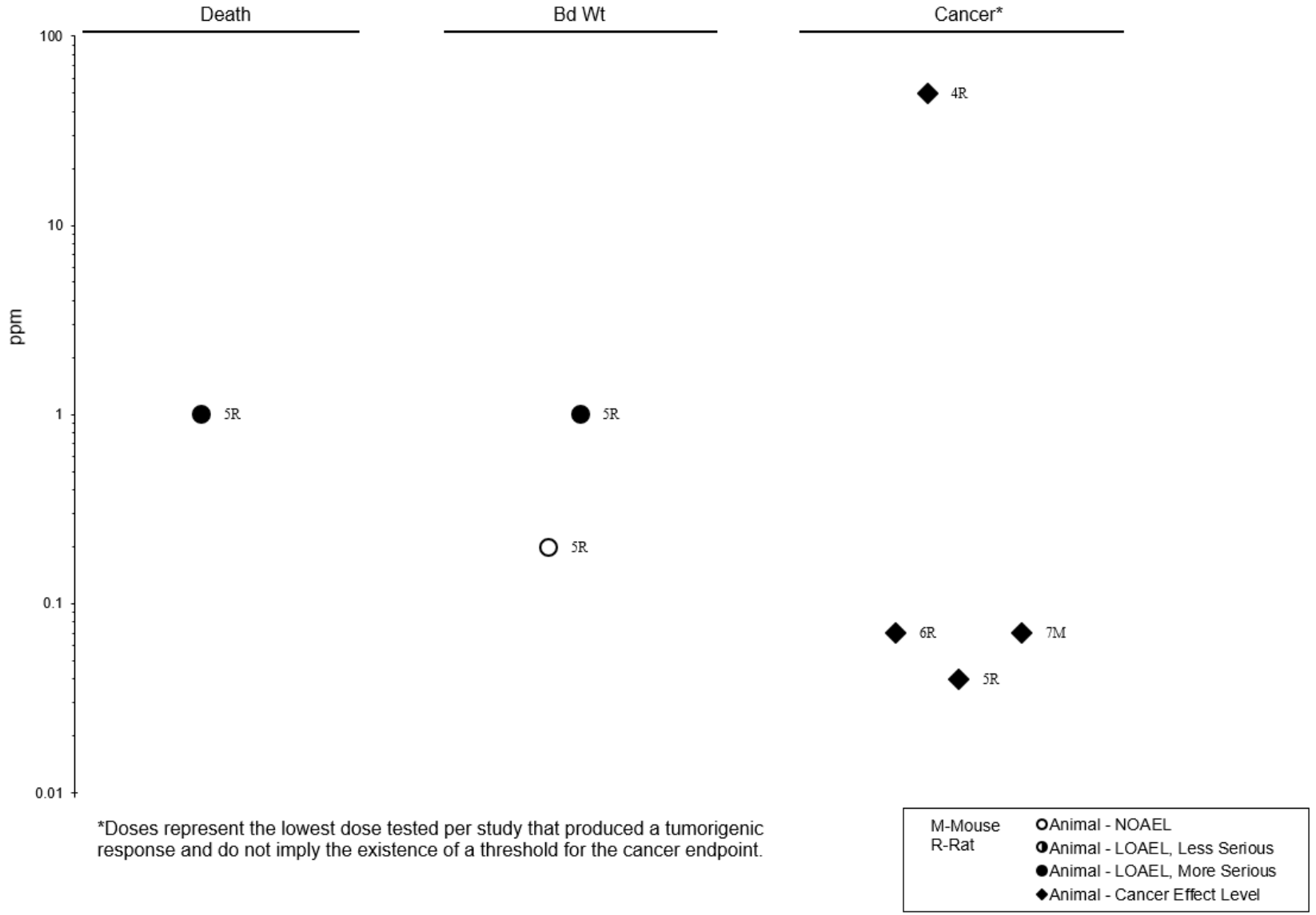


Figure 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Inhalation
Chronic (≥365 days)



*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.

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
1	Monkey (African green) 6 M	Once (G)	0, 50	LE, BW, OW, GN, HP, BC, CS	Bd wt	50			No difference in body weight gain
					Hepatic		50		Enlarged, cherry-red liver
Maduagwu and Bassir 1980									
2	Monkey (African green) 6 M	5–11 days, 1 time/day (G)	0, 5	LE, BW, OW, GN, HP, BC, CS	Death			5	3/6 died
					Bd wt	5			No difference in body weight gain
					Hepatic			5	Necrosis
Maduagwu and Bassir 1980									
3	Rat (F344/ Du Crj) 3 M	Once (G)	0, 20	BC, HP	Hepatic			20	2–7-fold increases in serum AST and ALT and focal necrosis 1–2 days after dosing
Asakura et al. 1998									
4	Rat (F344/ Du Crj) 3 M	14 days, 1 time/day (G)	0, 4	BC, HP	Hepatic			4	Focal necrosis
Asakura et al. 1998									
5	Rat (BD) NS	Once (G)	40	LE, CS	Death			40	LD_{50}
Druckrey et al. 1967									
6	Rat (Fischer-344) 3–5 M	Once (G)	37, 48.1, 62.5, 81.3	LE, GN, HP	Death			48.1	4/5 died at 48.1 mg/kg
Frank et al. 1990									

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
7	Rat (NS) 7–8 NS	Once	0, 10	BC	Hepatic		10		6-fold increase in serum ALT
Garland et al. 1988									
8	Rat (Crl:CD [SD]) 5 M	14 days (GW)	0, 1, 2, 4	LE, BW, OW, HP	Bd wt	2	4		14% lower body weight at termination
					Hepatic		1		Inflammatory cell infiltration
Hamada et al. 2015; Takashima et al. 2015									
9	Rat (albino) 25 NS	1 or 2 weeks, 7 days/week (F)	0, 3.75	HP	Hepatic			3.75	Hemorrhagic necrosis
Khanna and Puri 1966									
10	Rat (Sprague-Dawley) 7–9 M	Once (G)	0, 0.3, 0.7, 1.9, 5.1, 13.7, 37.0, 100	OW, HP, BC, BI	Hepatic	0.7	1.9	13.7	LOAEL: vacuolation Serious LOAEL: necrosis
Korsrud et al. 1973									
11	Rat (Wistar) 10 M	Once (G)	0, 50	BW, OW, GN, HP, BC, CS	Bd wt			50	16% body weight loss (compared to 10% gain in controls)
					Hepatic			50	Necrosis with hemorrhage into peritoneum
Maduagwu and Bassir 1980									
12	Rat (Wistar) 10 M	5–11 days, 1 time/day (G)	0, 5	LE, BW, OW, GN, HP, BC, CS	Death			5	3/10 died
					Bd wt			5	41% body weight loss (compared to 32% gain in controls)
					Hepatic			5	Necrosis
Maduagwu and Bassir 1980									

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
13	Rat (Wistar) 8 M	10 days, 7 days/week (W)	0, 0.002, 0.003	BI, HP	Hemato	0.003			No changes in bone marrow histopathology
					Hepatic	0.003			No changes in liver histopathology
					Immuno	0.003			No changes in spleen histopathology
Moniuszko-Jakoniuk et al. 1999									
14	Rat (Holtzman) 17–32 F	Once on GD 18 (GO)	0, 15, 20	LE, CS	Death			15	3/32 pregnant rats died at 15 mg/kg
Nishie 1983									
15	Rat (Holtzman) 21 F	Once (GO)	0, 15, 20	BW, BC, BI, OW, HP	Bd wt	20			
					Hepatic			15	Necrosis, glycogen depletion
					Endocrine	20			No change in thyroid weight or histopathology
Nishie 1983									
16	Rat (Holtzman) 6–22 F	Once on GDs 9, 12, 14, and 15 (20 mg/kg) or GD 16, 18, or 20 (15 or 20 mg/kg) (GO)	0, 15, 20	BW, BC, BI, OW, HP	Hepatic			15	Severe centrilobular damage (necrosis and glycogen depletion) in dams
					Endocrine	20			No change in thyroid weight or histopathology in dams
Nishie 1983									
17	Rat (Holtzman) 6–22 F	Once on GD 15 or 20 (GO)	0, 20	DX	Develop			20	12–18% decrease in mean fetal weight
Nishie 1983									
18	Rat (Wistar) 7 M	10 days, 7 days/week (W)	0, 0.002	BC	Hepatic		0.002		≥2-fold increases in serum AST, ALT, ALP, and GGT
Roszczenko et al. 1996a									

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
19	Rat (Wistar) 7 M	10 days, 7 days/week (W)	0, 0.0007, 0.0016, 0.0035	HE, BC, BI	Hepatic	0.0007[b]	0.0016		Decreased serum total and latent iron binding capacity; $BMDL_{1sd}$=0.0014.
Roszczenko et al. 1996b									
20	Rat (Wistar) 12–20 M	Once (G)	0, 8, 9, 10	HP, BC	Hepatic			8	Necrosis; serum ALT and AST increased 15- and 22-fold (respectively) in germ-free rats and 1.7- and 1.9-fold (respectively) in conventional rats
Sumi and Miyakawa 1983									
21	Rat (Wistar) 5–20 M	Once (G)	0, 40	OF	Death			40	All rats died by day 21
Waynforth and Magee 1974									
22	Mouse (A/JNCr) 50 M	Once (GW)	0, 1, 5	BW, WI, GN, HP	Bd wt	5			
					Cancer			5	CEL: lung tumors (at sacrifice 16 weeks after dosing)
Anderson et al. 1992a									
23	Mouse (CD-1) 3 M	2 weeks, 7 days/week (GW)	0, 2, 4, 7, 10	LE, BC, BI	Death			7	All animals died within 6 days
					Hepatic	2	4		2-fold increases in serum ALT and AST
Doolittle et al. 1987									
24	Mouse (Swiss) 18 M, 13 F	1 week, 7 days/week (W)	0, 10	LE, CS, HP	Death			10	Decreased survival (survival at week 10: 0/13 males and 12/18 females treated versus and 32/33 male and 36/36 female controls)
					Cancer			10 F	CEL: kidney and lung tumors
Terracini et al. 1966									
25	Guinea pig (Hartley) 10M	Once (G)	0, 50	LE, BW, OW, GN, HP, BC, CS	Bd wt			50	10% body weight loss (compared to 8% gain in controls)
					Hepatic			50	Hemorrhagic centrilobular necrosis
Maduagwu and Bassir 1980									

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
26	Guinea pig (Hartley) 10 M	5–11 days, 1 time/day (G)	0, 5	LE, BW, OW, GN, HP, BC, CS	Death			5	4/10 died
					Bd wt			5	14% body weight loss (compared to 17% gain in controls)
					Hepatic			5	Necrosis
Maduagwu and Bassir 1980									
27	Hamster (Golden) 5–20 M	1–14 days, 7 days/week (W)	0, 4	CS, GN, HP	Hepatic		4		Portal venopathy
Ungar 1984									
28	Cat (domestic) 6 M	5–11 days, 1 time/day (G)	0, 5	LE, BW, OW, GN, HP, BC, CS	Death			5	4/6 died
					Bd wt			5	21% body weight loss (compared to 13% gain in controls)
					Hepatic			5	Necrosis
Maduagwu and Bassir 1980									
29	Cat (domestic) 6 M	Once (G)	0, 50	LE, BW, OW, GN, HP, BC, CS	Death			50	2/6 died
					Bd wt			50	13% body weight loss (compared to 4% gain in controls)
					Hepatic			50	Ascites; severe hemorrhage into peritoneum
Maduagwu and Bassir 1980									
INTERMEDIATE EXPOSURE									
30	Monkey (African green) 6 M	30 days, 1 time/day (G)	0, 1	LE, BW, OW, GN, HP, BC, CS	Hepatic			1	Necrosis
Maduagwu and Bassir 1980									
31	Rat (albino) 6 NS	34–110 days, 7 days/week (F)	0, 5, 10, 20	BW, FI, GN, HP	Death			10	6/6 died between days 62 and 95; at 20 mg/kg/day, 6/6 died between days 34 and 37
					Bd wt			10	35% decrease in body weight
					Gastro			20	Hemorrhage

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic	5		10	Necrosis
Barnes and Magee 1954									
32	Rat (Fischer-344) 89–91 M	16 weeks, 7 day/week (W)	0, 0.000075, 0.00075, 0.0075, 0.075, 0.75	BW, BI, OW, GN	Bd wt	0.75			
					Hepatic	0.75			No change in absolute or relative liver weight
Fukushima et al. 2005									
33	Rat (Crl:CD [SD]) 5 M	28 days (GW)	0, 0.5, 1, 2	LE, BW, OW, HP	BW	2			
					Hepatic	1	2		Inflammatory cell infiltration
Hamada et al. 2015; Takashima et al. 2015									
34	Rat (Fischer-344) 12–19 M	8 weeks, 7 days/week (W)	0, 3.9	BW, OW, HP	Bd wt	3.9			
					Hepatic		3.9		Eosinophilic or mixed cell foci and hepatocellular nodules
Jang et al. 1990									
35	Rat (MRC) 15–30 M	30 weeks, 5 days/week (W)	0, 0.4, 2	HP	Cancer			0.4	CEL: liver tumors
Keefer et al. 1973									
36	Rat (albino) 25 NS	4, 8, or 12 weeks, 7 days/week (F)	0, 7.2	HP	Hepatic			7.2	Hemorrhagic necrosis
Khanna and Puri 1966									
37	Rat (Fischer-344) NS M and F	20–30 weeks, 2 days/week (GO)	0, 11.1 (M); 8.1, 13.2 (F)	LE, GN, HP	Death			13.2 F	Some (NS) animals died in 6th week
					Cancer			8.1 F 11.1 M	CEL: liver, lung, and kidney tumors
Lijinsky and Kovatch 1989									

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
38	Rat (Fischer-344) 20 F	30 weeks, 5 days/week (W)	0, 0.75, 1.8	HP	Death			0.75	Decreased survival
					Cancer			0.75	CEL: liver tumors
Lijinsky and Reuber 1984									
39	Rat (Wistar) 10 M	30 days, 1 time/day (G)	0, 1	LE, BW, OW, GN, HP, BC, CS	Hepatic		1		Vacuolation and congestion
Maduagwu and Bassir 1980									
40	Rat (albino) 5–10 M, 5–10 F	Up to 40 weeks, 7 days/week (F)	0, 3.9	BW, FI, GN, HP	Death			3.9	Decreased survival
					Hepatic			3.9	Hemorrhagic necrosis
					Cancer			3.9	CEL: liver tumors in 19/20
Magee and Barnes 1956									
41	Rat (Wistar) 8 M	30 or 90 days, 7 days/week (W)	0, 0.002, 0.003	BI, HP	Hemato			0.002	Bone marrow histopathology changes: focal necrosis; edema, degeneration; decrease in megakaryocytes and migration to vascular sinus; myelosclerosis after 90 days
					Hepatic			0.002	Degeneration, argyrophilic and collagenic fibers, and inflammatory infiltrations near portal biliary tract after 30 days; steatosis and parenchymatosis after 90 days
					Immuno		0.002		Splenic histopathology changes: megakaryocytes in red pulp; enhanced lymphatic "texture" after 90 days
Moniuszko-Jakoniuk et al. 1999									
42	Rat (SD) 6 M	15 days (GW)	0, 0.5, 2, 4	BW, BC, HE, HP	Hepatic	0.5		2	Centrilobular hepatocyte degeneration and fibrosis; inflammation of central vein and subscapular region
Rothfuss et al. 2010									

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
43	Rat (Wistar) 10–12 M	30 weeks, 7 days/week (W)	0, 1.5, 3.7	LE, BW, OW, HP	Death			1.5	8/12 died at 1.5 mg/kg/day and 6/12 died at 3.7 mg/kg/day
					Bd wt		3.7		10% decrease in terminal body weight
					Cancer			1.5	CEL: liver tumors
Takahashi et al. 2000									
44	Mouse (A/JNCr) 39–50 M	16 weeks, 7 days/week (W)	0, 0.12, 0.25, 1.2	HP, BW, WI	Bd wt	1.2			
					Cancer			1.2	CEL: lung tumors
Anderson 1988									
45	Mouse (CD-1) 10 F	≥100 days total (75 days premating, through Gestation and possibly lactation) (W)	0, 0.026	CS, GN, DX	Develop			0.026	Increased perinatal deaths (stillborn and neonatal)
Anderson et al. 1978									
46	Mouse (Swiss Cr:NIH(s)) 10–20 F	1–4 weeks, 7 days/week (W)	0, 5	BW, WI, GN, BI, HP	Hepatic			5	Hemorrhage (mild to moderate centrilobular) at all time points (1, 2, and 4 weeks)
Anderson et al. 1986									
47	Mouse (A/JNCr) 50 M	4 weeks, 7 days/week (W)	0, 1.2	BW, WI, GN, HP	Bd wt	1.2			
					Cancer			1.2	CEL: lung tumors
Anderson et al. 1992a									
48	Mouse (A/JNCr) 50 M	16–48 weeks, 7 days/week (W)	0, 0.25	BW, WI, GN, HP	Bd wt	0.25			
					Cancer			0.25	CEL: increased incidence or number of lung tumors after 32 or 48 weeks
Anderson et al. 1992a									

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
49	Mouse (RF/Un) 83–262 M	49 days, 7 days/week (W)	0, 1.8	CS, GN, HP	Death			1.8	Decreased survival (mean 15 versus 20.5 months in controls)
					Cancer			1.8	CEL: liver and lung tumors
Clapp and Toya 1970									
50	Mouse (RF/Un) 17–262 M	224 days, 7 days/week (W)	0, 0.40	CS, GN, HP	Cancer			0.4	CEL: lung tumors
Clapp and Toya 1970									
51	Mouse (RF/Un) 94–262 M	Lifetime (average 266 days), 7 days/week (W)	0, 0.91	CS, GN, HP	Death			0.91	Decreased survival (mean 12 months versus 20.5 months in controls)
					Cancer			0.91	CEL: liver and lung tumors
Clapp and Toya 1970									
52	Mouse (C3Hf) 17 M, 20 F	13 weeks, 7 days/week (W)	0, 1.2	HP	Death			1.2	Decreased survival
					Cancer			1.2	CEL: liver, lung
Den Engelse et al. 1974									
53	Mouse (CD-1) 15 F	4–17 weeks, 7 days/week (W)	0, 0.26, 1.3, 2.6, 5.3	HE, HP, BC, BW, GN, WI	Death			2.6	1/15 exposed to 2.6 mg/kg/day and 3/15 exposed to 5.3 mg/kg/day
					Hepatic			2.6	Ascites (10/13 between 30 and 120 days of exposure at 2.6 mg/kg/day and 14/14 by exposure day 30 at 5.3 mg/kg/day)
					Immuno	0.26	1.3		Markedly reduced humoral response to sheep red blood cells and inhibition of alloantigenic response of T-cells.
Desjardins et al. 1992									
54	Mouse (C3H/) 30 M	5 months, 7 days/week (F)	0, 5.26	HP	Death			5.26	Decreased survival
					Hepatic			5.26	Hemorrhage/necrosis
					Cancer			5.26	CEL: liver, lung, and kidney tumors
Takayama and Oota 1965									

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
55	Mouse (Swiss) 26 M, 19 F	38 weeks, 7 days/week (W)	0, 1	CS, HP	Death			1	Decreased survival (respective male and female survival to 30 weeks: 69 and 53% versus 94 and 89% in controls)
					Cancer			1	CEL: liver, lung, and kidney tumors
Terracini et al. 1966									
56	Guinea pig (Hartley) 10 M	30 days, 1 time/day (G)	0, 1	BW, OW, GN, HP, BC, CS	Hepatic			1	Necrosis
Maduagwu and Bassir 1980									
57	Hamster (Syrian Golden) 30–31 M	Up to 7 months 7 days/week (W)	0, 1.1	BW, WI, GN, HP	Death			1.1	3/30 died within 6 months and 27/30 died within 7 months
					Cancer			1.1	CEL: liver tumors
Bosan et al. 1987									
58	Hamster (Golden) 5–10 M	28 days, 7 days/week (W)	0, 4	GN, HP, CS	Hepatic		4		Portal venopathy
Ungar 1984									
59	Hamster (Golden) 4–13 M	8, 12, or 16 weeks, 7 days/week (W)	0, 4	GN, HP, CS	Death			4	Three animals died prior to week 8
					Hepatic		4		Portal venopathy after 8 weeks
					Cancer			4	CEL: liver tumors after 16 weeks
Ungar 1986									

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
60	Dog (Beagle) 6 or 8 M, F	24 weeks, 2 consecutive days/week (C)	0, 2	LE, BW, CS, BC, UR, HE, OW, GN, HP	Bd wt		2		Weight loss (up to 18%)
					Hepatic			2	Severe hepatic effects including increased serum enzymes (AST, ALT, ALP, GGT), bile acids, and bilirubin; histopathology (necrosis, inflammation, cholestasis, vacuolation, lobular collapse, fibrosis and biliary hyperplasia); ascites; hepatic encephalopathy; secondary effects on clotting parameters
Boothe et al. 1992									
61	Dog (Mongrel) 5–8 NS	4 weeks, 2 consecutive days/week (C)	0, 2.51	BC, HP	Hepatic			2.51	Hepatic necrosis, stromal collapse, fibrous structure; increased serum AST, ALT, and LDH (80, 220, and 94% compared to controls)
Hashimoto et al. 1989									
62	Dog (Mongrel) 9–11 NS	4 weeks, 2 consecutive days/week (C)	0, 2.51	BC, HP	Death			2.51	1/9 died of acute liver failure 2 weeks after dosing ended
					Hepatic			2.51	Extensive necrosis, stromal collapse, destruction of lobular architecture, inflammation, cirrhosis; increased serum ALP, AST, and bilirubin; ascites
Madden et al. 1970									

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
63	Cat (domestic) 6 M	30 days, 1 time/day (G)	0, 1	LE, BW, OW, GN, HP, BC, CS	Death			1	3/6 died between days 25 and 30
					Bd wt			1	28% body weight loss (compared to 26% gain in controls).
					Hepatic			1	Necrosis
Maduagwu and Bassir 1980									
64	Rabbit (New Zealand) 5 M	12 weeks, 7 days/week (GW) 0, 0.5		BC, BI, HP	Hepatic			0.5	Hepatocytic infiltration in portal areas, central vein congestion, red blood cell hemolysis, vacuolar degeneration
					Repro			0.5	Markedly reduced (96% less than controls) serum testosterone; increased serum estradiol; testicular histopathology (disorganized seminiferous tubules; interstitial edema; degeneration of germinal epithelium in seminiferous tubules and Sertoli cells; exfoliation of cells in lumen of tubules; blood vessel congestion; proliferation of Leydig cells)
Sheweita et al. 2017									
65	Mink ("pastel") 3 M	23–34 days, 7 days/week (F)	0, 0.32, 0.63	LE, BW, FI, HP, CS	Death			0.32	Decreased survival
					Hepatic			0.32	Necrosis
Carter et al. 1969									
66	Mink (NS) 12 M, 12 F	122 days, 7 days/week (F)	0, 0.04, 0.05, 0.06, 0.08, 0.13, 0.17	GN, CS, HP	Hepatic	0.08	0.13		Venopathy
Koppang and Rimeslatten 1976									

Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
CHRONIC EXPOSURE									
67	Rat (Wistar) 24 M, 24 F	96 weeks, 7 days/week (F)	0, 0.013, 0.13, 1.3	BW, OW, FI, WI, HE, BC, HP	Bd wt	1.3			
					Hepatic	0.013	0.13		Nodular hyperplasia
					Renal	1.3			
					Cancer			1.3	CEL: liver (both sexes);leukemia (females)
Arai et al. 1979; Ito et al. 1982									
68	Rat (Wistar) 60 M, 60 F treated, 240 M, 240 F control	1, 1.5, or 3.5 years, 7 days/week (W)	M: 0, 0.001, 0.003, 0.005, 0.011, 0.022, 0.044, 0.065, 0.087, 0.109, 0.131, 0.174, 0.218, 0.261, 0.348, 0.697 F: 0, 0.002, 0.005, 0.010, 0.019, 0.038, 0.076, 0.115, 0.153, 0.191, 0.229, 0.306, 0.382, 0.459, 0.612, 1.224	GN, LE, HP	Death			0.022 M 0.038 F	Decreased survival
					Cancer			0.022 M 0.038 F	CEL: liver tumors
Peto et al. 1984, 1991a, 1991b									
69	Rat (Wistar) 15 M	54 weeks, 7 days/week (F)	0, 0.5	HP	Hepatic	0.5			
					Cancer			0.5	CEL: testicular tumors
Terao et al. 1978									
70	Mouse (A/JNCr) 47–48 M	72 weeks, 7 days/week (W)	0, 0.24	BW, WI, GN, HP	Bd wt	0.24			
					Cancer			0.24	CEL: lung tumors
Anderson et al. 1992a									

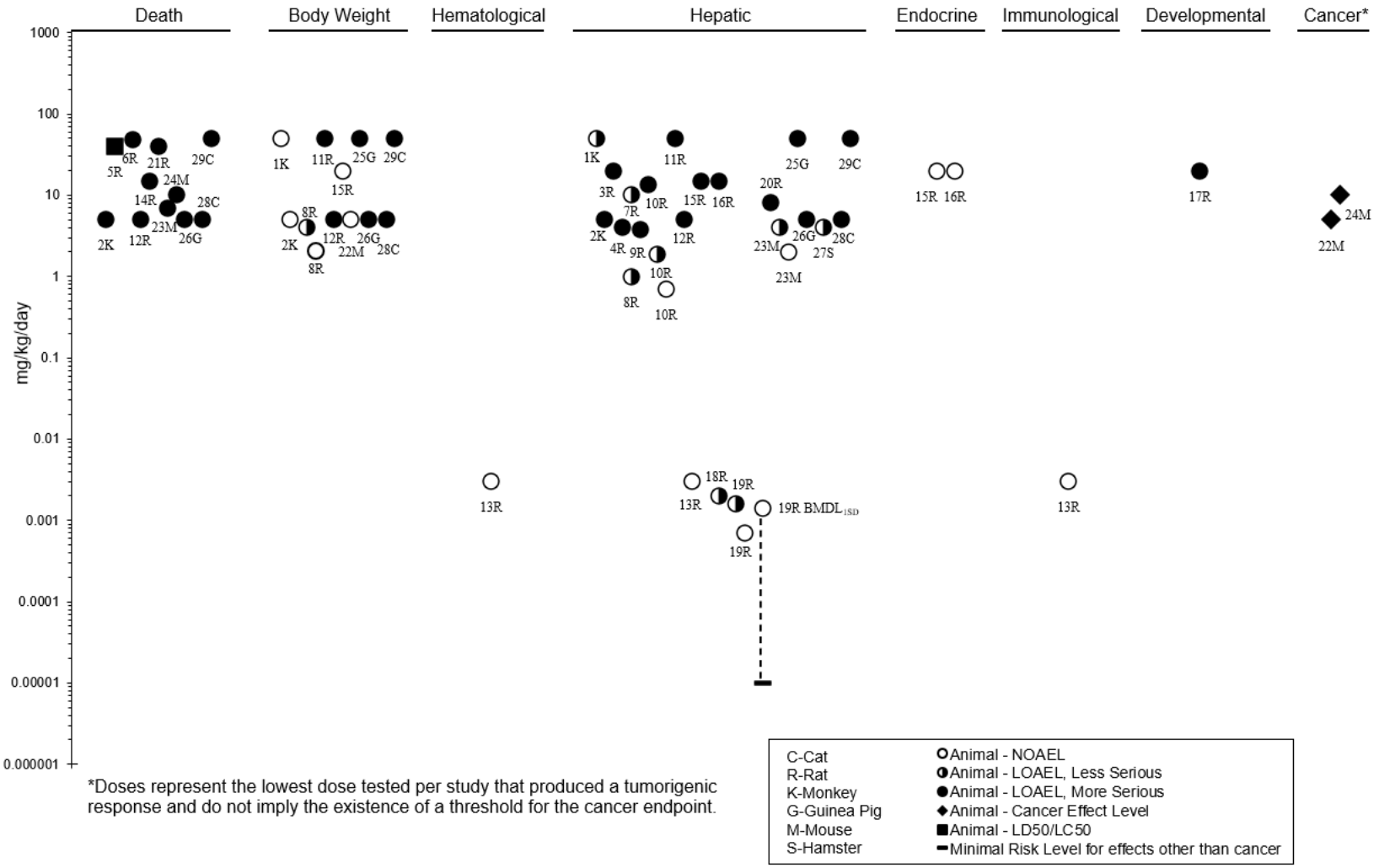
Table 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
71	Mouse (RF/Un) 47 M	Lifetime (average 406 days), 7 days/week (W)	0, 0.43	CS, GN, HP	Death			0.43	Decreased survival (mean 17 versus 20.5 months in controls)
					Cancer			0.43	CEL: liver and lung tumors
Clapp and Toya 1970									
72	Dog (mongrel) 6–10 M and F (number per sex NS)	56 weeks, 2 days/week (C)	0, 2	CS, OF, GN, HP	Bd wt			2	Intermittent anorexia and weight loss (2–15% of body weight).
					Hepatic			2	Fibrosis, centrilobular necrosis, cirrhosis, ascites; 13–54-fold increase in serum bile acids; 20–40-fold increase in sulfobromophthalein retention time
Butler-Howe et al. 1993									
73	Mink (NS) 6 M, 14 F	321–670 days, 7 days/week (F)	0, 0.1–0.13	GN, HP, CS	Death			0.1	Decreased survival
					Hepatic			0.1	Venopathy, focal necrosis
					Cancer			0.1	CEL: liver tumors
Koppang and Rimeslatten 1976									

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an acute-duration oral minimal risk level (MRL) calculated using benchmark dose analysis. The $BMDL_{1SD}$ of 0.0014 mg/kg/day was divided by an uncertainty factor of 100 (10 for human variability and 10 for animal to human extrapolation), resulting in an MRL of 0.00001 mg/kg/day ($1x10^{-5}$ mg/kg/day).

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical changes; $BMDL_{1SD}$ = 95% lower confidence limit on the BMD associated with 1 SD change from control mean; (C) = capsule; CEL = cancer effect level; CS = clinical signs; DX = developmental toxicity; (F) = feed; F = female(s); FI = food intake; (G) = gavage; (GO) = gavage in oil; (GW) = gavage in water; Gastro = gastrointestinal; GD = gestation day; GGT = gamma-glutamyl transferase; GN = gross necropsy; HE = hematology; HP = histopathological; Immuno = immunological; LD_{50} = dose producing 50% deaths; LDH = lactate dehydrogenase; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight; SD = standard deviation; UR = urinalysis; (W) = drinking water; WI = water intake

Figure 2-3. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral
Acute (≤14 days)



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

Figure 2-3. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral
Intermediate (15–364 days)

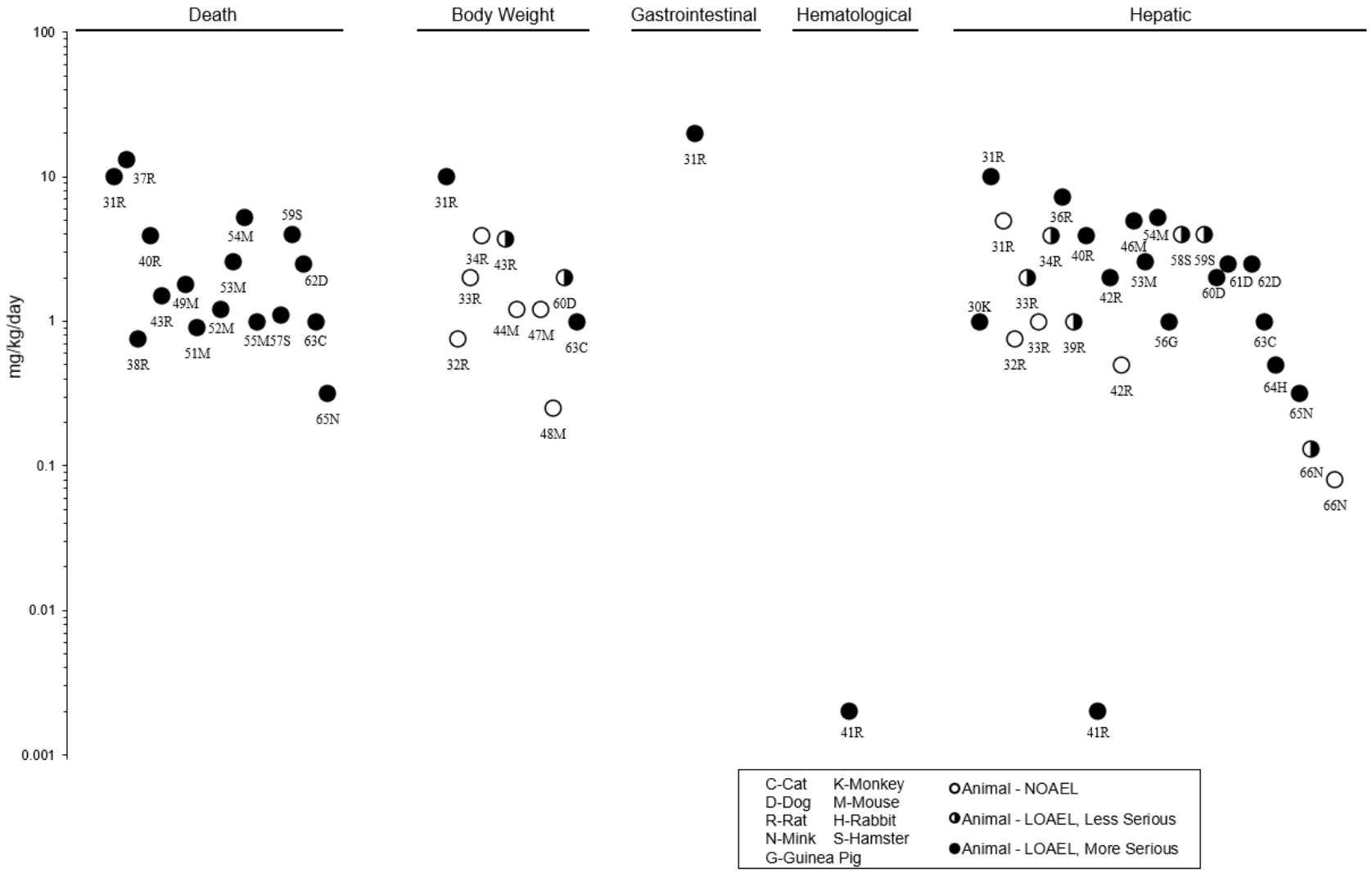


Figure 2-3. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral
Intermediate (15–364 days)

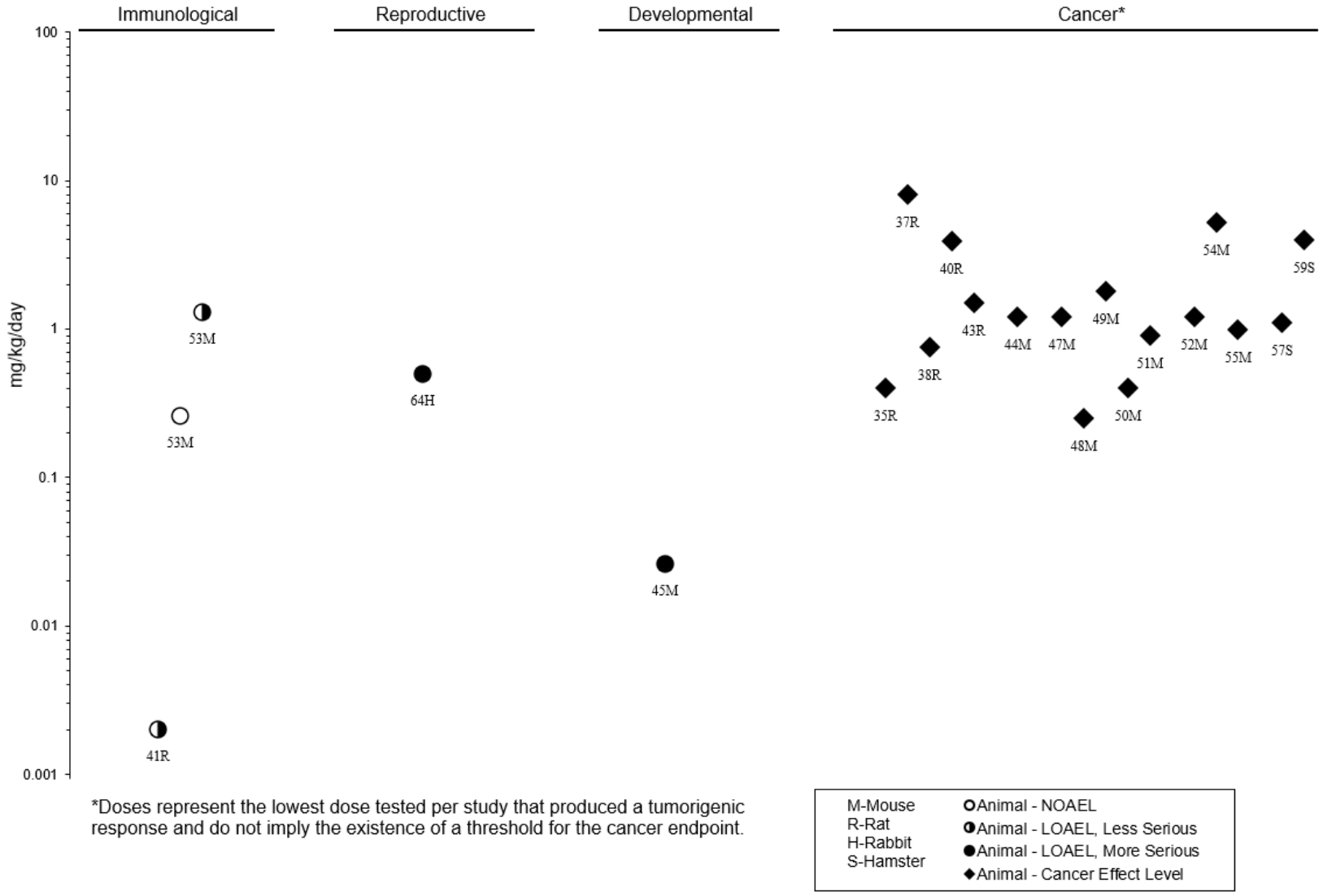
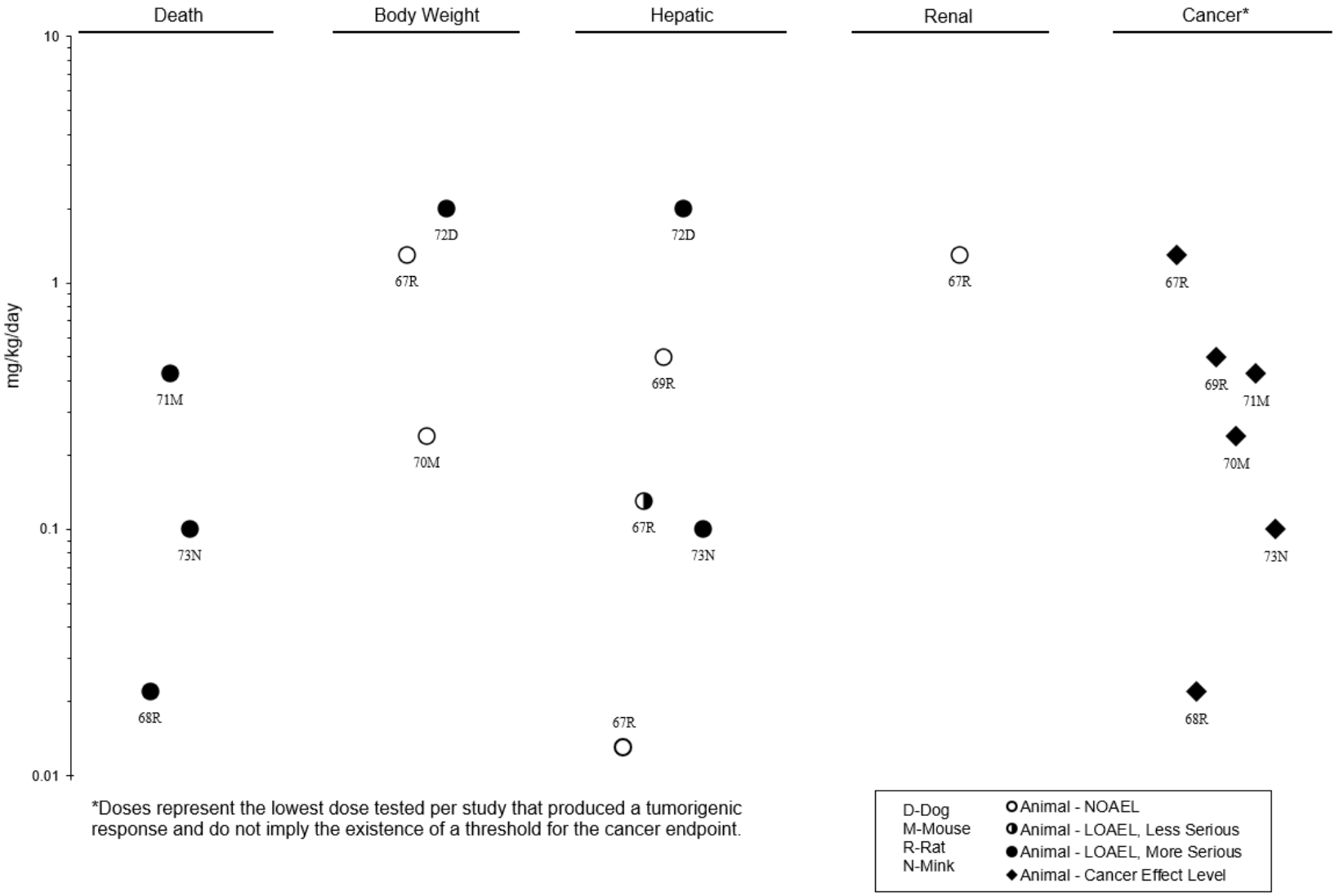


Figure 2-3. Levels of Significant Exposure to N-Nitrosodimethylamine – Oral
Chronic (≥365 days)



*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.

2.2 DEATH

At least two human deaths following inhalation of NDMA have been reported in the literature. One was a male chemist who was involved in the production of NDMA and was exposed to an unknown level of fumes for about 2 weeks, and subsequently to an unknown level of fumes during cleanup of a spilled flask (Freund 1937). The subject became ill 6 days later and showed abdominal distention, large amounts of yellow ascitic fluid, and a tender and enlarged liver and enlarged spleen. The subject died 6 weeks after the last exposure. The other death was that of a male worker who was exposed to unknown concentrations of NDMA in an automobile factory. Autopsy of this subject showed a cirrhotic liver with areas of regeneration (Hamilton and Hardy 1974).

At least three human deaths following oral exposure to NDMA have been reported in the literature. One of the fatalities was a woman who was apparently poisoned over a 2-year period by her husband (Fussgaenger and Ditschuneit 1980; Pedal et al. 1982). It was estimated by the authors that she received at least four doses as high as 250–300 mg each, for a total dose of <1.5 g. Both clinical and autopsy findings indicated that she died of liver failure. Two of five people who consumed lemonade tainted with unknown quantities of NDMA (an adult male and a l-year-old boy) died within days, while the other three people (an adult female, adult male, and 2.5-year-old girl) survived (Cooper and Kimbrough 1980; Kimbrough 1982). Based on animal studies, the authors estimated that the adult might have received about 1.3 g and the boy might have received about 300 mg. In both cases, clinical and autopsy findings primarily showed liver failure and cerebral hemorrhage.

The lethality of inhaled NDMA has been evaluated in several acute-duration studies with animals. Single 4-hour exposure LC_{50} values of 78 ppm (95% confidence limits of 68 and 90 ppm) and 57 ppm (95% confidence limits of 51 and 64 ppm) were determined for rats and mice, respectively (Jacobson et al. 1955). The observation time in these assays was 14 days. The cause of death was not specified, but liver damage and hemorrhage in various abdominal tissues were the predominant pathologic findings. Druckrey et al. (1967) reported that the "LD_{50}" for rats exposed to NDMA by inhalation for 1 hour is 37 mg/kg. The air concentration corresponding to this dose was not reported, but a value of 925 ppm can be calculated from information provided in the report; however, confidence in this value is low, because this information is ambiguously reported. Two of three dogs that were exposed to 16 ppm NDMA for 4 hours died or were moribund by the second day (Jacobson et al. 1955). All dogs that were similarly exposed to 43–144 ppm died or were moribund within 3 days.

Acute-duration oral studies of NDMA in animals have shown mortalities at single doses as low as 15 mg/kg and repeated doses as low as 5 mg/kg/day. Single-dose lethality studies have been conducted in which NDMA was administered to rats and cats by gavage. A dose of 10 mg/kg did not produce deaths in rats within 48 hours (Sumi and Miyakawa 1983). Single doses of 15 and 20 mg/kg were not lethal for nonpregnant rats, but mortalities were seen in pregnant rats treated once on gestation day (GD) 18 at these doses (3/32 at 15 mg/kg and 6/17 at 20 mg/kg) (Nishie 1983). The authors estimated an LD_{50} of ~23 mg/kg for pregnant rats based on these findings (Nishie 1983). Jenkins et al. (1985) reported that single 25 mg/kg doses of NDMA resulted in 100% mortality in an unspecified number of rats; it is not clear whether a control group was used in this study. A group of six male F344 rats survived a single dose of 37 mg/kg NDMA, while 48.1 mg/kg was lethal to 4/5 rats and higher doses were lethal to all animals (Frank et al. 1990). Druckrey et al. (1967) determined an LD_{50} of 40 mg/kg for rats using an unspecified graphic technique; confidence limits and specific mortality data were not reported. All 12 rats that were treated with a single dose of 40 mg/kg in a skin grafting (immunology) experiment died by day 21, but the stress of skin graft rejection may have contributed to mortality (Waynforth and Magee 1974). Two of six cats died when treated with 50 mg/kg (Maduagwu and Bassir 1980).

Administration of a daily dose of 4 mg/kg/day in the drinking water of hamsters for 1, 2, 4, 7, or 14 days did not result in mortality (Ungar 1984). Rats, guinea pigs, cats, and monkeys that were treated with NDMA by gavage at a dose of 5 mg/kg/day for 11 days experienced 30–40% mortality, with deaths occurring as early as 5 days (Maduagwu and Bassir 1980). All three mice given 7 mg/kg/day NDMA by gavage daily died within 6 days, while groups dosed at 2 or 4 mg/kg/day survived 2 weeks of treatment (Doolittle et al. 1987). Rats treated by gavage daily with 8 mg/kg/day NDMA for 6 days experienced 10% mortality within 1 month (McGiven and Ireton 1972); a control group was not evaluated in this study. Administration of NDMA in the drinking water at a daily dose of 10 mg/kg/day for 1 week resulted in decreased survival in mice (Terracini et al. 1966).

Deaths in rats and mice resulting from intermediate-duration oral exposure to NDMA were usually attributed to liver toxicity or carcinogenicity. In these studies, NDMA effects on survival were observed at doses as low as 0.32 mg/kg/day. In rats, decreased survival resulted when NDMA was given in the drinking water for 30 weeks at 0.75 mg/kg/day, 5 days/week (Lijinsky and Reuber 1984) or 1.5 mg/kg/day, 7 days/week (Takahashi et al. 2000). Decreased survival was also reported when rats received 6 mg/kg via gavage for 2 days/week for 30 weeks (Lijinsky et al. 1987); in this study, control animals were not included, but there was 100% mortality by 40 weeks after cessation of treatment. Barnes and Magee (1954) administered NDMA in the diet to small numbers of rats (6/group);

2.5 mg/kg/day produced no deaths, 5 mg/kg/day produced 100% mortality after 62–93 days and 10 mg/kg/day produced 100% mortality after 34–37 days. Rats treated with 3.9 mg/kg/day in the diet for 40 weeks also had high mortality (Magee and Barnes 1956). Jenkins et al. (1985) observed mortality in rats that received 2.5 mg doses of NDMA by gavage for 4 days/week for 9 weeks, but it is unclear if this is dose per kg body weight or per rat. Daily exposure to 1 mg/kg/day by gavage for 30 days had no effect on survival of rats (Maduagwu and Bassir 1980).

In intermediate-duration studies with mice, decreased survival resulted from treatment with doses of 1.8 mg/kg/day via drinking water for 49 days (Clapp and Toya 1970), 1.2 mg/kg/day via drinking water for 13 weeks (Den Engelse et al. 1974), 1 mg/kg/day via drinking water for 38 weeks (Terracini et al. 1966), 2.6 mg/kg/day in drinking water for at least 45 days (Desjardins et al. 1992), and 5.26 mg/kg/day via diet for 5 months (Takayama and Oota 1965). Survival was not affected in mice that received 0.4 mg/kg/day in drinking water for 32 weeks (Clapp and Toya 1970) or 1.3 mg/kg/day for 17 weeks (Desjardins et al. 1992).

Survival data for intermediate-duration oral exposure to NDMA are also available for cats, dogs, guinea pigs, monkeys, hamsters, and mink. Daily gavage exposure to 1 mg/kg for 30 days caused decreased survival in cats but not guinea pigs or monkeys (Maduagwu and Bassir 1980). Among nine mongrel dogs exposed for 4 weeks to capsules containing 2.51 mg/kg NDMA twice per week, one dog died of acute liver failure 2 weeks after the end of exposure (Madden et al. 1970); in other studies in mongrel or Beagle dogs, there were no mortalities for 4 (Hashimoto et al. 1989) or 24 weeks (Boothe et al. 1992) at the same dose and regimen. In hamsters, daily administration of 4 mg/kg/day in the drinking water for 8, 12, or 16 weeks resulted in occasional moribundity (Ungar 1986), while no lethality resulted from daily administration of the same dose for 28 days (Ungar 1984). Once weekly gavage treatment with a dose of 10.7 mg/kg for 4 weeks or 5.4 mg/kg for 20 weeks was lethal for hamsters (Lijinsky et al. 1987). Mink that were given doses of 0.32 or 0.63 mg/kg/day in the diet died after 23–34 days of treatment (Carter et al. 1969), but small numbers of animals were tested (three per dose). Mink fed a contaminated diet that provided approximately 0.18 mg/kg/day died within a 2-month period (Martino et al. 1988), but there is uncertainty about the dietary concentration of NDMA used to calculate the dose and the durations of exposure.

In chronic-duration studies of orally exposed rats, mice, and mink, decreases in survival have been reported, often attributable to cancers. Survival was not affected in 15 rats that received 0.5 mg/kg/day of NDMA in the diet for 54 weeks (Terao et al. 1978). However, in a large (60 rats/sex/dose), multi-dose

(15 nonzero dose levels) carcinogenicity bioassay of rats, Peto et al. (1984, 1991a, 1991b) observed dose-related decreases in survival due to liver tumors at doses ≥0.022 mg/kg/day in drinking water. Decreased survival was noted in mice exposed to 0.43 mg/kg/day in the drinking water for life (average 406 days) (Clapp and Toya 1970). In mink, mortality resulted from ingestion of 0.1 mg/kg/day in the diet for 321–670 days (Koppang and Rimeslatten 1976).

2.3 BODY WEIGHT

No data pertaining to NDMA-induced effects on body weights of humans exposed by any route were located, and no studies reporting body weight effects in animals exposed by dermal contact were located. A chronic study of female rats exposed via inhalation to concentrations of 0, 0.04, 0.2, or 1 ppm (4–5 hours/day, 4 days/week for ~72 weeks) reported lower body weight (~10% based on visual examination of data presented graphically) at the highest exposure level (Klein et al. 1989, 1991). At lower exposures, body weight decrements occurred, but did not reach 10% difference from controls until the animals reached advanced age (~3 years), and survivors were few.

In animals exposed orally, body weight effects were generally seen only in the context of severe liver toxicity or tumors. Body weight was not affected in female rats given a single dose of 15 or 20 mg/kg by gavage (Nishie 1983) or mice given a single dose of 1 or 5 mg/kg by gavage (Anderson et al. 1992a). Decreased body weight gain was reported in male rats after 15 days of exposure to NDMA at 2 mg/kg/day, and at 4 mg/kg/day, absolute body weight was also decreased (magnitude of change was not reported); severe liver effects accompanied the body weight changes (Rothfuss et al. 2010). In other experiments in mice exposed via drinking water, body weights were not affected by treatment for 4 or 16 weeks at 1.2 mg/kg/day or for 16–72 weeks at ~0.25 mg/kg/day (Anderson 1988; Anderson et al. 1992a). In rats exposed to NDMA in drinking water for 30 weeks, a 10% decrease in terminal body weight was observed at 3.7 mg/kg/day; however, there were mortalities and liver tumors at doses ≥1.5 mg/kg/day in this study (Takahashi et al. 2000). Khanna and Puri (1966) reported progressive body weight loss in rats given 7.2 mg/kg/day NDMA in drinking water for up to 12 weeks. The magnitude of body weight loss was not reported. These animals also exhibited severe hepatic effects (hemorrhagic necrosis throughout the lobule) (Khanna and Puri 1966). No body weight changes were reported in rats given 3.9 mg/kg/day NDMA in drinking water for 8 weeks (Jang et al. 1990) or up to 0.75 mg/kg/day for 16 weeks (Fukushima et al. 2005). Dogs exhibited body weight losses (up to 18%) when given capsules containing 2 mg/kg NDMA on 2 days/week for 24 or 56 weeks; the animals in these experiments exhibited severe liver effects at this dose (Boothe et al. 1992; Butler-Howe et al. 1993).

2.4 RESPIRATORY

An occupational epidemiology study reported increased odds of self-reported respiratory symptoms, including nose bleeds, burning or dry throat, hoarseness, and severe dry cough among 172 Swedish rubber production workers when compared with 118 unexposed subjects (Jönsson et al. 2009). Median breathing zone NDMA concentrations in the workplaces ranged between 0.24 and 8.2 μg/m^3 (Jönsson et al. 2009). Hidajat et al. (2019a) reported significantly increased subdistribution hazard ratios (SHRs) (based on competing risk survival analysis) for mortality from respiratory diseases with increasing cumulative NDMA exposure in a cohort of 36,442 U.K. rubber workers followed for 49 years. The SHR for the highest quartile of cumulative NDMA exposure was 1.41 (95% confidence interval [CI] 1.30, 1.53). Results were not adjusted for smoking status. The authors noted that confounding by unmeasured smoking status was unlikely (based on sensitivity analyses) but could not be ruled out entirely. In humans who expired from NDMA poisoning, autopsies showed hemorrhages in the bronchi, trachea, and/or lungs (Freund 1937; Kimbrough 1982); further details of the fatalities are reported in Section 2.2.

No studies evaluating respiratory effects in animals following inhalation or dermal exposure to NDMA were located. Macroscopic congestion was noted in the lungs of rats exposed to 3.75 mg/kg/day in the diet for 1–12 weeks (Khanna and Puri 1966). The severity of the congestion cannot be determined because results of lung histological examinations were not reported. No chronic-duration oral studies of respiratory effects in animals were located.

2.5 CARDIOVASCULAR

Higher risks of mortality from circulatory and cerebrovascular diseases and ischemic heart disease (SHRs up to 1.48) were reported in a large cohort of 36,441 male U.K. rubber factory workers followed for 49 years (Hidajat et al. 2020). Confounding by unmeasured smoking status could not be ruled out in this study (Hidajat et al. 2020). In cases of fatal exposure to NDMA, cardiovascular effects seen at autopsy included subpericardial hemorrhage (Freund 1937) and myocardial and endocardial bleeding (Kimbrough 1982). Additional details of these cases are provided in Section 2.2.

No studies were located regarding cardiovascular effects in animals following inhalation exposure to NDMA. Macroscopic congestion was noted in the myocardium of rats that were administered 3.75 mg/kg/day doses of NDMA in the diet for 1–12 weeks (Khanna and Puri 1966). The severity of the congestion cannot be determined because results of heart histological examinations were not reported. In rats given 0.2 mg/kg/day (presumably by gavage) daily for 2 weeks, alterations in blood levels of cardiovascular biomarkers were seen: creatine kinase MB activity was increased by 103% compared to controls, while homocysteine levels were decreased by 25% (Sheweita et al. 2014). No other cardiovascular endpoints were evaluated in the study.

2.6 GASTROINTESTINAL

Increased risks of digestive diseases with increasing NDMA exposure were reported among U.K. rubber industry workers in a 49-year follow-up study (Hidajat et al. 2019a). In this study, SHRs across quartiles of cumulative NDMA exposure ranged up to 1.60 (95% CI 1.31, 1.95). In humans who died from NDMA poisoning, autopsy findings included gastrointestinal hemorrhage (Freund 1937; Kimbrough 1982; Pedal et al. 1982); additional details of these cases are reported in Section 2.2. Studies of gastrointestinal effects in animals following inhalation or dermal exposure to NDMA were not located. After intermediate-duration oral exposure of animals, NDMA produced gastrointestinal effects. Barnes and Magee (1954) observed occasional hemorrhage into the gastrointestinal tract in rats that died from treatment with a single 50 mg/kg dose of NDMA by gavage, or with 10 mg/kg/day doses in the diet for 34–37 days. The numbers of animals examined were unspecified (single-dose study) or small (six in the diet study), and the frequency of occurrence was not indicated. Gastrointestinal hemorrhages were also observed in mink that ingested 0.32 or 0.63 mg/kg/day via diet for 23–34 days (Carter et al. 1969). Only three mink per dose were treated, the hemorrhages occurred in a total of three mink, and the dose(s) that the affected mink received was not specified. The cause of the hemorrhages in the mink was attributed to gastric and duodenal erosions.

Rostkowska et al. (1998) observed increases in the specific activities of several lysosomal exoglycosidases (N-acetyl-p-hexosaminidase, β-galactosidase, and α-mannosidase) in the gastrointestinal tracts of rats exposed for 10 or 90 days to NDMA in drinking water (20 μg/L). The study authors suggested that the increased enzyme activities could stem from macrophages recruited by damaged cells in the alimentary canal.

2.7 HEMATOLOGICAL

In five individuals poisoned with unknown amounts of NDMA in lemonade, slight to severe thrombocytopenia was reported (Kimbrough 1982). No other studies were located regarding hematological effects in humans exposed to NDMA or in animals exposed dermally to NDMA. Hematological evaluations were performed in dogs that were exposed to 16–144 ppm NDMA for 4 hours (Jacobson et al. 1955). After exposure at all concentrations, increased coagulation time, prothrombin time, and plasma cholinesterase levels occurred. In addition, leukopenia was observed at all exposure levels. The dogs exhibited severe liver toxicity and mortality (see Section 2.2) at these exposure levels; the hematological effects may have resulted from profound liver toxicity.

Administration of NDMA in drinking water to rats for 10 days resulted in dose-related increases in blood hemoglobin concentration at doses ≥0.0016 mg/kg/day; hematocrit was not affected, and other hematology parameters were not measured. In a corollary study by the same group of investigators (Moniuszko-Jakoniuk et al. 1999), rats exposed via drinking water to 0.002 or 0.003 mg/kg/day for 10 days exhibited no changes in bone marrow histopathology.

When rats were exposed to 4 mg/kg/day NDMA by daily gavage for 15 days, significant decreases in platelet and reticulocyte counts were observed in conjunction with serious liver damage (Rothfuss et al. 2010). After 30 and 90 days of drinking water exposure to NDMA, rats showed increased blood hemoglobin concentrations at 0.0016 mg/kg/day (17–28%); however, hemoglobin concentration was significantly decreased (9%) after 30 days of exposure to 0.0035 mg/kg/day (Roszczenko et al. 1996b). Hematocrit was significantly decreased (10% less than controls) at the high dose in the 30-day experiment (a 90-day experiment at the high dose was not conducted) and no other parameters were measured. Moniuszko-Jakoniuk et al. (1999) reported bone marrow histopathology changes in rats exposed to NDMA in drinking water for 30 or 90 days. After 30 days at 0.003 mg/kg/day and after 90 days at both 0.002 and 0.003 mg/kg/day, bone marrow changes included including focal necrosis of bone marrow, edema, degeneration, decrease in bone marrow megakaryocytes and migration to vascular sinus, and myelosclerosis (Moniuszko-Jakoniuk et al. 1999). Macroscopic congestion was noted in spleens of rats that were administered 3.75 mg/kg/day doses of NDMA in the diet for 1–12 weeks (Khanna and Puri 1966). The severity of the congestion cannot be determined because results of spleen histological examinations were not reported.

2.8 MUSCULOSKELETAL

No studies were located regarding musculoskeletal effects in humans or animals following inhalation, oral, or dermal exposure to NDMA.

2.9 HEPATIC

A large cohort mortality study of 36,144 male U.K. rubber factory workers reported an increased risk of mortality from liver disease for workers in the third quartile of cumulative NDMA exposure (SHR 2.22; 95% CI 1.24, 3.99) (Hidajat et al. 2020). In the highest quartile, the SHR was elevated (1.35) but the CI included 1.0. No attempt to adjust for alcohol intake was made.

In a cohort of 2,875 German female rubber workers with occupational exposure to nitrosamines, the rate of mortality from non-alcoholic cirrhosis of the liver was elevated compared with the rate in the general population of German women (Straif et al. 1999). All 10 of the cases of non-alcoholic cirrhosis occurred among women employed in production of technical rubber goods, and the risk of death from this cause increased with earlier year of hire and longer duration of employment in rubber good production (Straif et al. 1999). Straif et al. (1999) reported that the highest documented nitrosamine concentration in the facilities included in their study was NDMA at 170 μg/m^3. The study authors did not report concentrations of other nitrosamines in the women's workplaces; however, the other primary nitrosamine measured in rubber production facilities is N-nitrosomorpholine, which often occurs at exposure levels similar to NDMA (de Vocht et al. 2007; Hidajat et al. 2019b; Jönsson et al. 2009; Straif et al. 2000; Tricker et al. 1989).

Four cases of liver disease in humans resulting from inhalation exposure to NDMA have been described in the literature. Two of the subjects died; these cases are discussed in Section 2.2. Of the subjects who survived, one was a chemist who was exposed to unknown concentrations of fumes and experienced exhaustion, headache, cramps in the abdomen, soreness on the left side, nausea, and vomiting for at least 2 years (Freund 1937). The second case was an automobile factory worker who was exposed to unknown levels of NDMA and became violently ill with jaundice and ascites (Hamilton and Hardy 1974). Five members of a family who consumed unknown quantities of NDMA in lemonade became ill with nausea, vomiting, and serum chemistry changes associated with acute liver disease, as well as generalized bleeding and slight to severe thrombocytopenia (Cooper and Kimbrough 1980; Kimbrough 1982). As indicated in Section 2.2, two of these people died; the other three were released from a hospital 4–21 days

after admission. Another fatality due to ingestion of NDMA was attributed to liver failure (Fussgaenger and Ditschuneit 1980; Pedal et al. 1982). Autopsies of the subjects described above showed that the primary effects were hemorrhagic and cirrhotic changes in the liver and necrosis and hemorrhage in other internal organs. Barnes and Magee (1954) briefly described two cases of liver cirrhosis among three men using NDMA in a research laboratory for about 10 months. In one, cirrhosis was discovered at autopsy after the man died from bronchopneumonia. In the second, cirrhosis was discovered during follow-up for an unrelated operation. The latter patient showed improved liver function after 3 months with no exposure to NDMA (Barnes and Magee 1954).

Hepatotoxicity was reported at lethal concentrations of NDMA in dogs exposed by inhalation. Pathologic examination of dogs following exposure to 16–144 ppm NDMA for 4 hours showed marked necrosis and varying degrees of hemorrhage in the liver (Jacobson et al. 1955). Related effects at all concentrations included increased bilirubin levels and increased sulfobromophthalein retention.

Hepatotoxicity of NDMA has been investigated in numerous oral studies of acute, intermediate, and chronic duration in several animal species. Hepatotoxicity is the most prominent and characteristic systemic effect of NDMA, resulting in centrilobular necrosis, hemorrhage, fibrosis, cirrhosis, and ascites. In acute studies, these characteristic hepatotoxic alterations were seen in rats following single gavage doses as low as 8–20 mg/kg (Asakura et al. 1998; Barnes and Magee 1954; Nishie 1983; Sumi and Miyakawa 1983) and following daily doses of ~4 mg/kg in the diet or via gavage for 1 or 2 weeks (Asakura et al. 1998; Hamada et al. 2015, 2022; Khanna and Puri 1966; Takashima et al. 2015). Jenkins et al. (1985) observed degenerative alterations collapse of reticulum network in the centrilobular areas followed by regeneration in the livers of rats following a single 2.5 mg/kg gavage dose of NDMA; however, a control group was not reported. The alterations were nonnecrotic and did not result in loss of the lobular architecture. After single gavage doses in rats, nonnecrotic histologic alterations (clumping and slight vacuolation of cells in the central vein area) occurred at 1.9 mg/kg and no alterations occurred at 0.7 mg/kg (Korsrud et al. 1973). Hepatocellular hypertrophy was observed in mice administered nitrosamines daily by gavage for 4 days. However, because results were not reported for individual compounds, it was unclear whether NDMA induced hypertrophy in this study (Nishie et al. 1972). After 14 daily gavage doses of NDMA, rats exposed to doses ≥1 mg/kg/day exhibited focal inflammatory cell infiltration in the liver, and at the highest dose of 4 mg/kg/day, effects on the liver included single cell necrosis, anisokaryosis, and increased mitotic figures (Hamada et al. 2015; Takashima et al. 2015). In studies that examined serum chemistry changes, marked increases in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and/or gamma-glutamyl

transferase (GGT) were observed (Asakura et al. 1998; Doolittle et al. 1987; Garland et al. 1988; Roszczenko et al. 1996a). Daily gavage exposure to 5 mg/kg for 5–11 days produced hemorrhagic necrosis in rats, guinea pigs, cats, and monkeys; this dose was lethal in all species tested (Maduagwu and Bassir 1980). Hamsters that ingested daily doses of 4 mg/kg/day in the drinking water for 1, 2, 4, 7, or 14 days showed portal venopathy (Ungar 1984).

A series of acute- and intermediate-duration studies in rats exposed to low concentrations of NDMA in drinking water was conducted by the same group of investigators (Moniuszko-Jakoniuk et al. 1999; Roszczenko et al. 1996a, 1996b). In these studies, groups of 7–8 male Wistar rats were exposed for 10, 30, or 90 days to concentrations of 10–50 µg/L (0.0007–0.0035 mg/kg/day). Each individual study evaluated limited endpoints, but taken together, the studies demonstrate liver effects at low doses after both acute and intermediate durations. After 10 days of exposure, doses of 0.0016–0.002 mg/kg/day resulted in effects on iron indices (decreased total and latent iron binding capacity) and serum enzymes (≥2-fold increases in AST, ALT, ALP, and GGT) (Roszczenko et al. 1996a, 1996b), but no liver histopathology changes at doses up to 0.003 mg/kg/day (Moniuszko-Jakoniuk et al. 1999). After 30 days of exposure to ≥0.0016 mg/kg/day, similar perturbations of iron indices were observed, and serum enzyme levels remained increased (Roszczenko et al. 1996a, 1996b). In addition, there was evidence for serious liver histopathology changes at 0.002 and 0.003 mg/kg/day, including degeneration, argyrophilic and collagenic fibers, and inflammatory infiltrations near portal biliary tract after 30 days (Moniuszko-Jakoniuk et al. 1999). The effects increased in severity to include steatosis and parenchymatosis after 90 days (Moniuszko-Jakoniuk et al. 1999).

In other intermediate-duration studies with rats exposed to higher doses, characteristic hepatic effects (as described above) were produced by treatment with NDMA in the diet at doses of ≥2 mg/kg/day for 15 days (Rothfuss et al. 2010), 7.2 mg/kg/day for 4–12 weeks (Khanna and Puri 1966), 10 mg/kg/day for 62–95 days (Barnes and Magee 1954), and 3.9 mg/kg/day for 40 weeks (Magee and Barnes 1956). No liver histopathology changes were observed at 0.5 mg/kg/day for 15 days (Rothfuss et al. 2010). Inflammatory cell infiltration in the liver was observed at low incidence in rats given 28 daily gavage doses of 2 mg/kg/day; no other effects were reported (Hamada et al. 2015; Takashima et al. 2015). Jenkins et al. (1985) observed cirrhosis in rats that received 2.5 mg doses of NDMA by gavage for 4 days/week for 9 weeks, but it is unclear if this is dose per kg body weight or per rat. A dose of 1 mg/kg/day administered by gavage for 30 days produced centrilobular congestion and vacuolation of hepatocytes without necrosis in rats (Maduagwu and Bassir 1980). Hepatic alterations were not observed in rats treated with 5 mg/kg/day in the diet for 110 days (Barnes and Magee 1954). Preneoplastic lesions

(eosinophilic or mixed-cell foci or glutathione-S-transferase placental type positive [GST-P+] foci) in the liver were observed in rats given 3.9 mg/kg/day NDMA in drinking water for 8 weeks (Jang et al. 1990) or 0.075 mg/kg/day in water for 16 weeks (Fukushima et al. 2005). In a drinking water study in rats exposed to a very low dose of NDMA (0.002 mg/kg/day), 2–4-fold increases in ALT, ALP, and/or GGT (compared to controls) were observed after 30 or 90 days; however, liver histopathology was not examined, so the severity of the observed liver toxicity is uncertain (Roszczenko et al. 1996a).

In mice, hemorrhagic necrosis was observed in the livers after doses of 5 mg/kg/day in the drinking water for 1–4 weeks (Anderson et al. 1986) or ≥5.26 mg/kg/day in the diet for at least 5 months (Takayama and Oota 1965). In an immunotoxicity study, Desjardins et al. (1992) observed ascites, presumably resulting from hepatotoxicity, in mice exposed to 2.6 mg/kg/day NDMA in drinking water for 4–17 weeks.

Liver effects resulting from intermediate-duration oral exposure have been observed in species other than rat and mouse. Treatment with 1 mg/kg/day by gavage for 30 days was hepatotoxic for guinea pigs, cats, and monkeys (Maduagwu and Bassir 1980). Dogs given NDMA by capsule at 2–2.5 mg/kg/day on 2 days/week for 4–24 weeks exhibited profound hepatic injury including necrosis, cholestasis, fibrosis, cirrhosis, lobular collapse, and ascites (Boothe et al. 1992; Hashimoto et al. 1989; Madden et al. 1970; Strombeck et al. 1983). Central vein congestion, erythrocyte hemolysis, and vacuolar degeneration were seen in rabbits given 0.5 mg/kg/day NDMA by daily gavage for 12 weeks (Sheweita et al. 2017). Fibrotic and proliferative alterations without necrosis or hemorrhage were observed in rabbits treated with an average NDMA dose of 1.6 mg/kg/day in the diet for 22 weeks; this experiment did not include a control group (Magee and Barnes 1956). Occlusive alterations in the portal veins developed in hamsters that received daily 4 mg/kg doses in the drinking water for 28 days or 8, 12, or 16 weeks (Ungar 1984, 1986). Similar hepatic venopathy occurred in mink exposed to 0.13–0.15 mg/kg/day in the diet for 122 days (Koppang and Rimeslatten 1976). Mink that were given doses of 0.32 or 0.63 mg/kg/day in the diet for 23–34 days had widespread liver necrosis (Carter et al. 1969), but low numbers of animals were tested (three per dose). Liver necrosis was also observed in mink that ingested 0.18 mg/kg/day via diet (Martino et al. 1988); however, interpretation of this study is limited by uncertainty regarding exposure duration and concentration.

The only chronic oral study of NDMA that used more than one dose was Peto et al. (1984, 1991a, 1991b); these authors conducted a large cancer dose-response study in rats and reported limited information on nonneoplastic changes. Groups of 60 rats/sex were exposed to 1 of 15 concentrations of NDMA in drinking water (between 0.033 and 16.896 ppm) for 3.5 years. These water concentrations yielded

estimated doses from 0.001 to 0.697 mg/kg/day (as reported in Peto et al. 1984, 1991b). Controls (240/sex) received untreated water. Groups of six rats/sex/dose were sacrificed after 12 and 18 months, and the remaining animals were observed until natural death, moribund appearance, or appearance of palpable liver abnormalities. Macroscopic examinations were performed on all animals. Histopathology examinations were performed on grossly observed lesions; apart from these, only the liver and esophagus (target for N-nitrosodiethylamine, which was also tested) were routinely examined microscopically. Results for the interim sacrifices were not reported separately. In both male and female rats, NDMA doses ≥0.022 mg/kg/day were associated with decreased survival due to liver tumors. Significant dose-related trends were observed for several nonneoplastic or preneoplastic liver lesions, including hyperplastic nodules, cytomegaly, cysts, hepatocyte shrinkage (males only), and abnormality of glycogen-containing cells (females only). However, statistically significant increases in the incidence of these nonneoplastic changes (either individually or grouped) were seen only at doses ≥0.022 mg/kg/day. Because both increased liver tumor incidence and reduced survival due to tumors were observed at the same doses (≥0.022 mg/kg/day), neither a NOAEL nor a LOAEL for noncancer endpoints can be identified from these data.

In other chronic-duration studies, hepatotoxic effects were not observed in rats that were treated with 0.5 mg/kg/day NDMA in the diet for 54 weeks and then observed untreated for 15 weeks (Terao et al. 1978). At an early interim sacrifice (after 5 weeks of exposure) of only one animal per group, the liver from the exposed animal exhibited proliferation of the smooth endoplasmic reticulum under electron microscopy; at the terminal sacrifice, however, no histopathologic changes were reported in the liver. It is possible that any adverse effects on the liver were partially reversed during the post-exposure recovery period. Necrosis, fibrosis, cirrhosis, and ascites were seen in mongrel dogs given 2 mg/kg NDMA by capsule 2 days/week for 56 weeks (Butler-Howe et al. 1993). Liver injury in mink that ingested 0.1 mg/kg/day doses of NDMA in the diet for 321–670 days consisted of occlusive changes in the hepatic veins with focal necrosis (Koppang and Rimeslatten 1976).

Mechanisms of Hepatotoxicity. NDMA treatment in dogs and rats has been used as a model for human liver fibrosis (and its sequelae of cirrhosis, portal hypertension, and hepatocellular carcinoma) for nearly 40 years. As a result, a great deal of research has been performed to investigate the molecular mechanisms and pathophysiology of NDMA-related hepatic effects. George et al. (2019) published a succinct review of this research, detailing the effects of NDMA and its metabolites on hepatic cell populations. As discussed therein and summarized briefly below, NDMA induces liver effects through inflammation and oxidative stress mediated by metabolites.

As discussed further in Section 3.1.3, NDMA is rapidly metabolized in the liver by microsomal membrane-bound cytochrome P450 (CYP) 2E1 to form hydroxymethylnitrosamine, which then undergoes nonenzymatic degradation to formaldehyde and the reactive methyldiazonium ion. Downstream metabolites of these compounds include methanol and the methyl carbonium ion. Several of these metabolites are known to be potent hepatotoxicants. The methyldiazonium ion is a potent alkylating agent that methylates deoxyribonucleic acid (DNA) and proteins, resulting in damage to hepatic tissues. In addition, formaldehyde is an electrophilic molecule that reacts with a wide range of macromolecules, including proteins. Protein alkylation and cross-linking is a candidate molecular initiating event leading to hepatic fibrosis in NDMA-exposed organisms.

NDMA metabolites induce fibrosis through interactions with hepatocytes, lymphocytes, and sinusoidal endothelial cells (George et al. 2019). Both formaldehyde and methanol induce inflammation in the liver, leading to hemorrhagic necrosis. Generation of reactive oxygen species results, exacerbating the injury to hepatocytes and leading to lymphocyte release of proinflammatory cytokines (e.g., transforming growth factor β1 and nuclear factor-κB) and activation of Kupffer cells. Oxidative stress and lipid peroxidation deplete hepatic antioxidants and antioxidant enzymes including catalase and glutathione peroxidase.

Injury to endothelial cells results in the release of fibrogenic mediators including fibroblast growth factor-1 and connective tissue growth factor as well as induction of hedgehog signaling (promotes hepatic regeneration). In addition, release of Factor VIII (a blood-clotting protein also known as anti-hemophilic factor) from injured endothelial cells may result in aggregation of platelets, which triggers further production of inflammatory (transforming growth factor β1) and fibrogenic (platelet-derived growth factor) mediators. Fibrogenic cytokines are also released from activated Kupffer cells, leading to activation of resting stellate cells. The activated stellate cells produce collagen and other connective tissue proteins in an effort to repair the injured liver. Deposition of collagen fibrils in the extracellular matrix leads to fibrosis, cirrhosis, and portal hypertension. In addition to the DNA damage induced by reactive intermediates of NDMA metabolism, repeated injury and repair induced by these metabolites may also be involved in the mechanism of liver cancer from NDMA exposure (George et al. 2019).

2.10 RENAL

No studies were located regarding renal effects in humans following any exposure to NDMA or in animals exposed by inhalation or dermal application.

Limited information is available regarding renal effects of orally administered NDMA in animals. In a study by Nishie (1983), pregnant and nonpregnant rats were treated with a single NDMA dose of 15 or 20 mg/kg/day by gavage. An unspecified number of deceased animals (dose and pregnancy state not indicated) had distal tubule necrosis two days following treatment, while surviving rats had normal kidneys. Macroscopic congestion was noted in kidneys of rats that were administered 3.75 mg/kg/day doses of NDMA in the diet for 1–12 weeks (Khanna and Puri 1966). The severity of the congestion cannot be determined because results of kidney histological examinations were not reported. Moderate tubule congestion and other effects (glomerulus dilatation, slightly thickened Bowman's capsule) were observed in mink that ingested 0.18 mg/kg/day via diet (Martino et al. 1988); limitations of this study include uncertainty regarding exposure duration and dietary concentration.

2.11 DERMAL

No studies were located regarding dermal effects in humans or animals following inhalation or oral exposure to NDMA. Small ulcerations and scarring of the skin were observed in hairless mice that were treated once weekly with topical doses of 33.3 mg/kg for 20 weeks (Iversen 1980).

2.12 OCULAR

In a group of 172 Swedish rubber industry workers exposed to NDMA concentrations up to 8.4 $\mu g/m^3$, odds of self-reported itching, runny, or burning eyes were increased when compared with 118 unexposed subjects (Jönsson et al. 2009). No other studies reporting ocular effects in humans exposed to NDMA were identified in the literature searches. No studies were located regarding ocular effects in animals following oral or dermal exposure.

Little information is available regarding ocular effects of inhaled NDMA. Doolittle et al. (1984) reported reddened eyes in rats exposed to 500 or 1,000 ppm for 4 hours. As noted in Section 2.2, acute exposure to much lower concentrations of NDMA was lethal to rats, mice, and dogs. The lack of mortality in rats at the higher concentrations in the Doolittle et al. (1984) study may be attributable to the fact that the animals were killed immediately following exposure and not observed for subsequent death.

2.13 ENDOCRINE

Adrenal relative weight and mitotic count were increased in rats following a single 20 mg/kg gavage dose of NDMA (Nishie 1983). Histological examinations of the adrenal glands were not described. There was no effect on thyroid weight or histology in the same study.

2.14 IMMUNOLOGICAL

One study of immune system markers in humans exposed to NDMA during work in rubber production (Jönsson et al. 2009) was located; no other immunological studies in humans were identified. In a group of 172 Swedish rubber industry workers exposed to NDMA and several other nitrosamines, blood levels of eosinophils and immunoglobulin G (IgG) were significantly increased (14 and 11%, respectively) when compared with 118 unexposed subjects. There were no significant differences in leukocyte or neutrophil counts or in levels of α1-antitrypsin, C-reactive protein, or IgA, IgM, or IgE. Across the eight facilities where the exposed workers were employed, median detected breathing zone concentrations of NDMA ranged between 0.24 and 8.2 $\mu g/m^3$ (Jönsson et al. 2009). Other nitrosamines, including N-nitrosomorpholine, N-nitrosodiethylamine, N-nitrosodi-n-butylamine, N-nitrosopiperidine, and N-nitrosopyrrolidine, were detected less frequently and at lower concentrations.

No studies of immunological effects in animals following inhalation or dermal exposure to NDMA were located. Information regarding immunological effects of orally administered NDMA in animals is limited but demonstrates splenic histopathology changes and immune suppression after intermediate-duration exposure.

In a single dose study, skin graft survival time and white blood cell count were not reduced in rats after a 40 mg/kg dose of NDMA by gavage. All of the animals died by day 21, possibly due to the stress of skin graft rejection in addition to NDMA toxicity (Waynforth and Magee 1974).

Effects on splenic histology (megakaryocytes in red pulp and enhanced lymphatic "texture") were observed in rats exposed for 90 days to NDMA in drinking water at doses of 0.002 or 0.003 mg/kg/day; there were no changes after 30 days at either dose (Moniuszko-Jakoniuk et al. 1999). Desjardins et al. (1992) investigated humoral and cellular immune responses in mice following exposure to NDMA in the drinking water (0.26–5.3 mg/kg/day) for 30–120 days. Doses ≥2.6 mg/kg/day resulted in deaths and hepatotoxicity as evidenced by peritoneal ascites. Immunoglobulin M (IgM) antibody response to sheep

red blood cells (SRBCs) was significantly reduced at doses ≥1.3 mg/kg/day after 90 days of treatment and at 2.6 mg/kg/day after 120 days of treatment. Cellular immune response, monitored by allogeneic stimulation of cells in mixed lymphocyte reaction (MLR), was also suppressed at doses ≥1.3 mg/kg/day NDMA after 90 days of treatment and at 2.6 mg/kg/day after 120 days of treatment. No changes in immunological parameters were noted at 0.26 mg/kg/day. In other groups exposed for 90 days and then maintained without exposure for 30 days, immune suppression was reversed at 1.3 mg/kg/day, but not at 2.6 mg/kg/day (Desjardins et al. 1992).

2.15 NEUROLOGICAL

No studies were located regarding neurological effects in humans exposed to NDMA or in animals exposed via inhalation or dermal contact. Dogs treated with 2.5 mg/kg/day by capsule on 2 consecutive days/week for 3 weeks reportedly experienced marked central nervous system (CNS) depression; however, these effects were not further characterized (Strombeck et al. 1983). As these dogs developed liver necrosis and hepatic insufficiency, it is possible that the CNS depression was secondary to liver damage rather than a direct neurological effect of NDMA.

2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans following inhalation, oral, or dermal exposure to NDMA; studies in animals are limited to two intermediate-duration oral studies.

When male New Zealand rabbits were given daily gavage doses of 0.5 mg/kg/day NDMA for 12 weeks, marked reductions in serum testosterone were noted (81 and 96% less than controls at 8 and 12 weeks, respectively) along with variable increases in plasma estradiol (152 and 27% at 8 and 12 weeks) (Sheweita et al. 2017). At sacrifice at the end of exposure, testicular histopathology changes in treated rabbits included disorganized seminiferous tubules, interstitial edema, degeneration of germinal epithelium in seminiferous tubules and Sertoli cells, exfoliation of cells in lumen of tubules, blood vessel congestion, and proliferation of Leydig cells (incidences not reported, but effects not seen in controls). Biochemical analyses of the testes showed that the pathological changes accompanied a significant increase in oxidative stress (more than doubling of free radical thiobarbituric acid reactive substances [TBARS]), and depletion of antioxidant enzyme activities (glutathione, glutathione S-transferase, superoxide dismutase, and catalase) and 17 β-hydroxysteroid dehydrogenase (steroidogenic enzyme) (Sheweita et al. 2017).

There was no significant increase in time-to-conception in mice that were exposed to 0.026 mg/kg/day via drinking water for 75 days prior to mating (Anderson et al. 1978). Other reproductive indices were not evaluated.

2.17 DEVELOPMENTAL

No data pertaining to developmental effects in humans exposed to NDMA or in animals exposed via inhalation or dermal contact were located.

Acute-duration oral exposure of pregnant rats to NDMA has resulted in fetal mortality. A single 30 mg/kg dose administered by gavage on various GDs between 1 and 15 resulted in fetal mortality (Aleksandrov 1974; Napalkov and Alexandrov 1968). In addition, NDMA reportedly caused fetal deaths in rats when administered in the diet at a dose of 5 mg/kg/day beginning in early pregnancy (specific day and treatment duration not indicated) (Bhattacharyya 1965), by gavage at a dose of 2.9 mg/kg/day during the first or second weeks of gestation (Napalkov and Alexandrov 1968), or by gavage at a dose of 1.4 mg/kg/day throughout gestation until GDs 17–21 (not further specified) (Napalkov and Alexandrov 1968).

Teratogenic effects were not eva1uated in the studies of Nishie (1983) and Bhattacharyya (1965). Although Aleksandrov (1974) and Napalkov and Alexandrov (1968) evaluated these endpoints and observed no effect of NDMA treatment, confidence in the studies is low as these studies provided insufficient information regarding experimental design and results. Deficiencies in these studies include lack of control data, lack of maternal toxicity data, use of pooled data, and/or uncertain treatment schedule.

Fetuses of rats that received single 20 mg/kg doses of NDMA by gavage on GDs 15 or 20 had significantly decreased body weights. However, fetal survival data were not reported, and this dose was toxic to the dams as indicated by reduced body weight, hepatotoxicity, and mortality (Nishie 1983).

Intermediate-duration exposure of mice to NDMA in drinking water (0.026 mg/kg/day) for 75 days prior to mating and then during pregnancy and lactation resulted in a significant increase in neonatal mortalities (20% compared with 10% in controls) (Anderson et al. 1978). The deaths in treated offspring were equally distributed between stillbirths (19/185 versus 5/182 controls) and deaths up to postnatal day

(PND) 2 (19/186 versus 13/182). The incidence of litters with deaths was higher in the treated group (11/20) than in the controls (8/20) but the difference was not statistically significant. In one litter, all of the offspring died; the number of offspring in the litter was not reported.

2.18 OTHER NONCANCER

No other noncancer effects were reported in the NDMA literature.

2.19 CANCER

Overview. Evaluations of the carcinogenicity of NDMA by HHS (NTP 2021), EPA (IRIS 1987), and IARC (1987) have concluded that NDMA is "reasonably anticipated to be" or "probably" a human carcinogen, based primarily on robust evidence in animals. Human epidemiological data on the association between NDMA exposure and cancer, while extensive, are limited by numerous potential confounding factors, including challenges in estimating dietary intake of NDMA and its precursors, variations in endogenous formation of NDMA and uncertainties in the factors influencing such formation, and co-exposures to other carcinogenic agents. In contrast, there are abundant data showing the carcinogenicity of orally administered NDMA in acute-, intermediate-, and chronic-duration studies with rats, mice, hamsters, and mink. In addition, NDMA has been shown to induce mutations via metabolic activation in a multitude of *in vitro* and *in vivo* assays.

Endogenous Production of NDMA. NDMA is produced endogenously in the human body via nitrosation of amine and nitrate precursors in the stomach and other tissues (Hrudey et al. 2013). Estimates of the amount of NDMA produced endogenously in humans vary widely, depending on precursor intake as well as a variety of other factors (see Section 3.1), but available information suggests that for most people, endogenous formation is the largest source of exposure to NDMA (Hrudey et al. 2013). Most of the human studies of NDMA and cancer examined exposure to exogenous sources of NDMA (in food or drugs, or in the workplace) without considering the impact of differences in endogenous formation. Because of the significant contribution of endogenous formation of NDMA to human exposure levels, there is potential for misclassification of exposure in the human epidemiological studies, which would bias the results toward the null (no association).

Some epidemiological studies of NDMA and cancer have included estimates of endogenous N-nitroso compound formation using iron or heme-iron intake as a proxy (Jakszyn et al. 2006; Keszei et al. 2013).

These estimates are based on correlations between iron or heme-iron intake and total fecal excretion of N-nitroso compounds in humans (Jakszyn et al. 2006), so the findings are not specific to NDMA. In a European cohort study, Jakszyn et al. (2006) observed an association between iron intake and non-cardia adenocarcinoma of the stomach among subjects with *Helicobacter pylori* infection or low plasma vitamin C, but not uninfected persons or those with normal vitamin C levels. Keszei et al. (2013) observed a significant association between heme-iron intake and esophageal squamous cell carcinoma incidence among men in a large cohort study in the Netherlands. No association was observed in females, or among males or females in analyses of esophageal or gastric adenocarcinomas.

Epidemiological Studies. Available epidemiological data pertaining to cancers associated with exogenous NDMA exposure include occupational studies, studies of drugs contaminated with NDMA, and studies of dietary exposure to NDMA. Only one occupational epidemiology study (Hidajat et al. 2019a) identified in the literature reported associations between cancer and exposure to NDMA itself.

Hidajat et al. (2019a) followed 36,441 male employees in the United Kingdom rubber industry from 1967 to 2015 (total of 880,794 person-years). For these workers, exposure was likely to have been primarily via inhalation. Job information for each employee was available for 1967, and the authors assumed that the employees stayed in the same department and remained employed until retirement, death, or emigration. Exposure to NDMA was evaluated using a quantitative job-exposure matrix based on historic exposure measurements in the industry. Cases were determined based on underlying cause of death on death certificates. SHRs (comparable to Cox proportional hazard ratios) were estimated using competing risk survival analysis for quartiles of cumulative NDMA exposure, as follows: quartile 1 (Q1): <3.12 year $\mu g/m^3$; Q2: 3.12–5.96 year $\mu g/m^3$; Q3: 5.96–9.67 year $\mu g/m^3$; and Q4: >9.67 year $\mu g/m^3$. A lag time of 15 years was assumed in the models.

Cumulative NDMA exposure was associated with increased risks for several tumor types (Hidajat et al. 2019a). Results showed exposure-related linear trends in SHRs for bladder (up to 2.82 in Q4), stomach (up to 1.72 in Q4), leukemia (up to 3.47 in Q4), multiple myeloma (up to 2.81 in Q4), prostate (up to 5.36 in Q4), and liver (up to 2.86 in Q4). In addition, increased SHRs (without exposure-related trends) were observed in subjects of the fourth cumulative exposure quartile for brain (SHR=2.5), lung (1.7), NHL (2.25), esophagus (3.04), and pancreas (2.6). Cumulative exposures to N-nitrosomorpholine, total nitrosamines, and/or rubber dust and fumes were also associated with mortality from one or more of the cancer types for which an association with NDMA was observed.

This study (Hidajat et al. 2019a) had a number of strengths, including large cohort size with lengthy (49-year) follow-up and quantitative cumulative exposure estimates based on historic exposure measurements. The limitations noted by the study authors were: (1) the subject's individual employment histories prior to 1967 and during follow-up were not available (suggesting the possibility of exposure misclassification), (2) the 15-year lag time assumed in the analysis may not be suitable for blood cancers with shorter lag times; (3) some cancers may have been undercounted due to the use of underlying cause of death on death certificates; (4) information on confounders such as smoking history was not available for the subjects; (5) there was potential for selection bias because only workers who lived to 35 years of age were eligible for inclusion; (6) measurement error in individual exposure assessment was possible due to the use of a job-exposure matrix; and (7) there were correlations between NDMA and other exposures in the industry (other nitrosamines, nitrosomorpholine, rubber dust and fumes), as well as the possibility of cross-contamination across departments. These limitations make it difficult to establish clear associations between NDMA exposure and mortality from specific cancers.

There is a substantial number of studies of cancer in workers in the rubber industry, and these data formed the basis for the IARC classification of rubber industry work as carcinogenic to humans (IARC 1982, 1987). In addition to nitrosamines, rubber industry workers may be exposed to a wide range of other chemicals with known or potential carcinogenicity, including aromatic compounds, chlorinated compounds, metals, and others (IARC 1982). NDMA is known to be one of two primary nitrosamine exposures in this industry, the other being N-nitrosomorpholine (de Vocht et al. 2007; Hidajat et al. 2019b; Jönsson et al. 2009; Straif et al. 2000; Tricker et al. 1989). The large database of cancer studies in rubber industry workers was evaluated in a meta-analysis by Boniol et al. (2017). With the more recent study published by Hidajat et al. (2019a) that assessed NDMA specifically, the meta-analysis provides a synopsis of the relevant data from epidemiology of cancer in rubber industry employees.

Boniol et al. (2017) conducted a comprehensive meta-analysis of cancer associations with employment in rubber manufacturing, using the IARC definition for exposure to rubber manufacturing. These authors reviewed 234 publications and selected 46 cohort and 59 case-control studies for inclusion. Boniol et al. (2017) excluded case-control studies of nitrosamine exposure that were not specific to the rubber industry; thus, nitrosamine exposures in other industries, which may not include exposure to NDMA, were excluded. In addition, Boniol et al. (2017) cross-referenced studies that reported results for the same cohort, ensuring that the studies of a given cancer in the same cohort were not included multiple times in the analysis. Summary relative risk estimates were estimated using a random effects model.

Boniol et al. (2017) evaluated studies of 32 individual cancer sites. Their analysis showed increased summary relative risks for bladder cancer (1.36, 95% CI 1.18, 1.57), leukemia (1.29, 95% CI 1.11, 1.52), cancers of the lymphatic and hematopoietic systems not otherwise specified (1.16, 95% CI 1.02, 1.31), and cancer of the larynx (1.46, 95% CI 1.10, 1.94). A borderline increased summary relative risk was calculated for lung cancer (1.08, 95% CI 0.99, 1.17); risks for other cancer sites were not increased. Significant heterogeneity between studies was observed for all of the above cancer sites except for unspecified cancers of the lymphatic and hematopoietic systems. The increased risks for bladder cancer, lung cancer, and leukemia were not changed when the trim and fill method to correct potential publication bias was applied to the data. Stratification of studies by participants' date of first employment showed that there were no increases in risks for bladder cancer, lung cancer, or leukemia among workers who began work after 1960, although the numbers of studies of recent employment were small.

In July 2018, NDMA contamination was discovered in some batches of the drug, Valsartan (an angiotensin II receptor antagonist used to treat hypertension and heart failure) (see Sections 5.5 and 5.6 for further information on NDMA contamination of medications). Since that time, two large cohort studies (Gomm et al. 2021; Pottegard et al. 2018) investigated whether use of NDMA-contaminated Valsartan was associated with cancer risk. Pottegard et al. (2018) and Gomm et al. (2021) conducted similarly designed cohort studies in which subjects using Valsartan were identified using national health and prescription registries (in Denmark and Germany, respectively). Both studies employed manufacturer and lot number data from the registries to identify subjects exposed to the contaminated batches. The cohort in the study by Pottegard et al. (2018) consisted of 5,150 people followed for a median of 4.6 years. Gomm et al. (2021) followed 409,183 subjects who were exposed to NDMA-contaminated Valsartan and 372,688 subjects who were not for 3.25 years. Neither study observed a significant increase in overall cancer risk or risk of specific cancer types, with the exception of a slight increase in liver cancer risk reported by Gomm et al. (2021) (hazard ratio [HR] 1.16, 95% CI 1.03, 1.31). When Gomm et al. (2021) categorized exposure into dose categories, however, there was no evidence for a dose-response relationship between liver cancer incidence and exposure to NDMA-contaminated Valsartan.

Strengths of both studies include use of nationwide registries of Valsartan prescriptions, limiting potential selection and recall biases. In addition, the large size of the cohort in the study by Gomm et al. (2021) provides substantial statistical power to detect an effect. Both studies controlled for covariates including age, sex, exposures to other medications, and comorbidities, but did not control for smoking or dietary intake of NDMA or its precursors. Importantly, the brief follow-up time (3.25 and 4.6 years in Gomm et

al. [2021] and Pottegard et al. [2018], respectively) is a significant limitation of both studies. This follow-up time is inadequate for most cancer types, which have much longer latency times.

The finding of NDMA contamination in ranitidine and nizatidine (drugs used to block stomach acid) in 2019 also prompted a number of epidemiological studies of cancer. Unlike the contamination of Valsartan, the source of the NDMA in ranitidine and nizatidine was not traced to a specific manufacturer. As a consequence, cohort studies of exposure to NDMA in these drugs have relied on referent groups composed of people prescribed other drugs to block stomach acid (H2 blockers like cimetidine and famotidine or proton pump inhibitors like omeprazole). The use of referent groups with exposure to other types of drugs introduces additional confounding into the analysis, because other drugs may modify the risk of cancer. For example, some epidemiological studies have reported an association between use of proton pump inhibitors (PPIs) and increased risk of stomach cancer (reviewed by Cheung and Leung 2019; Moon et al. 2019).

As shown in Table 2-3, the cohort studies of cancer among users of ranitidine and/or nizatidine (Adami et al. 2021; Iwagami et al. 2021; Kim et al. 2021a, 2021b; Nørgaard et al. 2022; Yoon et al. 2021) generally found no positive association with cancers of the stomach, colorectum, liver, kidney, breast, or pancreas. Adami et al. (2021) reported a significant increase in adenocarcinomas of the esophagus among ranitidine users compared with users of cimetidine, famotidine, or PPIs; in contrast, Kim et al. (2021b) reported a decreased incidence of esophageal cancer in ranitidine users compared with users of omeprazole (PPI) or famotidine.

In cohort studies, an increase in bladder cancer was associated with ranitidine use when the referent group consisted of PPI users (Nørgaard et al. 2022) but not when the referent group was users of other H2 blockers (Nørgaard et al. 2022; Yoon et al. 2021). A case-control study of 3,260 bladder cancer cases in Scotland reported a significant trend for increased odds of higher ranitidine use among cases after adjustment for smoking, age, comorbidities, and other medication use (Cardwell et al. 2021). The trend was seen when exposure categories were based on estimated daily doses or prescription numbers. In analyses of other acid blocking medications (cimetidine, other H2 receptor agonists apart from ranitidine, or PPIs), there was no association with bladder cancer (Cardwell et al. 2021). Unlike the cohort studies, this case-control study was not limited by potential confounding from exposure to other acid-blocking drugs. In addition, while many case-control studies are subject to recall bias (when exposures are assessed by subject questionnaire), Cardwell et al. (2021) used prescriptions in a database of general practice medical records to assess exposure.

Table 2-3. Overview of Epidemiological Studies of Ranitidine Use and Cancers

Reference (location)	Study type and population size	Follow-up time (years)	Case identification	Cancer site	Results[a]
Adami et al. 2021 (Denmark)	Cohort: 103,565 adult new users of ranitidine; compared with 182,497 adults who first used cimetidine or famotidine, and 807,725 first-time users of PPIs	14	Cancer Registry	Stomach	↔
				Esophagus	↑ (adenocarcinoma)
				Liver	↔
				Pancreas	↔
Iwagami et al. 2021 (Japan)	Cohort: 113,745 adult new users of ranitidine or nizatidine compared with 503,982 new users of other H2 blockers	2.4 or 2.3	Administrative insurance claims database	Stomach	↔
				Colorectal	↔
				Breast	↔
Kim et al. 2021a (South Korea)	Cohort: 132,629 adults using ranitidine at least 30 days, compared with 13,629 controls and 13,629 users of other H2 blockers	5	National health claims database	Stomach	↔
Kim et al. 2021b (United States)	Cohort: 582,028 adults prescribed ranitidine compared with 2,179,048 prescribed omeprazole and 909,970 prescribed famotidine	Up to 10	Private electronic medical record database (IBM®Explorys)	Stomach	↓
				Esophagus	↓
				Colorectal	↓
				Liver	↓
				Pancreas	↓
McGwin 2020 (United States)	Cohort: 13,856 ranitidine users compared with 128,107 users of PPIs or other H2 blockers	7	Cancer reports to FDA Adverse Event Reporting System	Stomach	↑
				Esophagus	↑
				Colorectal	↑
				Liver	↑
				Pancreas	↑
				Pharynx	↑
Nørgaard et al. 2022 (Denmark)	Cohort: 31,393 adult first-time users of ranitidine compared with 65,384 first-time users of other H2 blockers and 509,849 first-time users of PPIs	14, 15, and 11	Cancer Registry	Bladder	↑ (compared with PPI users but not users of H2 blockers)
				Kidney	↔

Table 2-3. Overview of Epidemiological Studies of Ranitidine Use and Cancers

Reference (location)	Study type and population size	Follow-up time (years)	Case identification	Cancer site	Results[a]
Yoon et al. 2021 (South Korea)	Cohort: 40,488 adult users of ranitidine compared with 10,122 famotidine users	7	National health claims database	Stomach	↔
				Colorectal	↔
				Liver	↔
				Bladder	↔
				Kidney	↔
				Breast	↔
Cardwell et al. 2021 (Scotland)	Nested case-control: 3,260 cases and 14,037 controls	Not applicable	Prescriptions in medical records	Bladder	↑

[a]↑: significant association (confidence limits for the effect estimate do not include 1.0); ↔: no significant association (confidence limits for the effect estimate include 1.0).

FDA = U.S. Food and Drug Administration; H2 = histamine receptor 2; PPI = proton pump inhibitor

McGwin (2020) evaluated whether there was a higher rate of cancer reports to the FDA's Adverse Event Reporting System (AERS) among adverse events reported for ranitidine compared with adverse events reported for other acid-blocking medications. The study authors reported increases in the reporting of cancers of the stomach, esophagus, colorectum, liver, pancreas, and pharynx among ranitidine adverse events compared with the referent groups. However, it should be noted that this study shows only that there was increased reporting of cancers and does not demonstrate increased incidence of cancers. Reports to the AERS may be submitted by consumers, physicians, lawyers, or others, and media reports on the ranitidine recall could have influenced submissions. For example, McGwin (2020) reported the numbers of adverse events by year from 2012 to 2020 and observed a marked increase (more than double) in adverse events from 2017 to 2018, when NDMA contamination in ranitidine was first discovered. A similar phenomenon was reported by Cohen Sedgh et al. (2021), who observed a marked increase in the reporting of Valsartan-related cancers to the FDA AERS after the recall date and associated media attention.

A total of 18 studies examining associations between NDMA exposure in the diet and cancer were located in the literature searches; Table 2-4 provides an overview of these studies. There is substantial uncertainty in the exposure estimates from dietary intake studies, due to variability in NDMA concentrations in foods, variability in the intake of NDMA precursors, and uncertainty regarding factors influencing endogenous formation of NDMA. With few exceptions (LaVecchia et al. 1995; Michaud et al. 2009; Pobel et al. 1995), these studies controlled for tobacco use, a significant additional source of NDMA exposure and confounding factor for some cancer types. Similarly, potential confounding by alcohol intake was considered in most studies, with the exception of Knekt et al. (1999) and La Vecchia et al. (1995).

Many of the epidemiological studies have focused on cancers of the gastrointestinal tract, and especially gastric cancers. A meta-analysis published in 2015 (Song et al. 2015) showed an increased risk of gastric cancer associated with NDMA exposure. The authors selected eight articles comprising 11 studies: seven were cohort studies (Jakszyn et al. 2006; Keszei et al. 2013; Knekt et al. 1999; Larsson et al. 2006 [four cohorts consisting of men and women with cardia and noncardia adenocarcinomas]) and four were case-control studies (De Stefani et al. 1998; La Vecchia et al. 1995; Palli et al. 2001; Pobel et al. 1995). These studies are summarized in Table 2-4. Song et al. (2015) calculated the relative risk of gastric cancer and NDMA intake (comparing high versus low) by random-effects model to be 1.34 (95% CI 1.02–1.76). Significant heterogeneity was observed in the studies of NDMA. There was no evidence of publication

Table 2-4. Overview of Epidemiological Studies of N-Nitrosodimethylamine Dietary Intake and Cancers

Cancer site	Citation (location)	Study type and population size	Food and beverage intake questionnaire	Case identification	Results[a]
Stomach	Keszei et al. 2013 (Netherlands)	Case-control: 497 cases of gastric noncardia adenocarcinoma and 166 cases of gastric cardia adenocarcinoma, 4,032 control men and women; mean follow-up 14.3 years	150 items; administered at baseline	Cancer registry, pathology confirmed	↑ for noncardia adenocarcinoma in men; ↔ for cardia adenocarcinoma or for either type in women
	Loh et al. 2011[b] (United Kingdom)	Cohort: 23,363 men and women; 64 cases; mean follow-up 11.4 years	131 items; administered at baseline	Cancer registry	↔
	Jakszyn et al. 2006 (Europe)	Cohort: 153,447 men and 368,010 women; 314 cases; mean follow-up 6.6 years	Number of items not reported; administered at baseline	Cancer registries, pathology confirmed	↔
	Larsson et al. 2006 (Sweden)	Cohort: 61,433 women; 156 cases; mean follow-up 18 years	67 or 97 items; administered at baseline	Cancer registries	↑
	Knekt et al. 1999 (Finland)	Cohort: 9,985 men and women; 68 cases; maximum follow-up 24 years	Number of items not reported; administered at baseline	Cancer registry	↔
	Palli et al. 2001 (Italy)	Case-control: 382 cases and 561 population-based controls	181 items	Hospital recruitment, pathology confirmed	↔
	De Stefani et al. 1998 (Uruguay)	Case-control: 340 cases and 698 hospital-based controls	Number of items not reported	Hospital recruitment	↑
	La Vecchia et al. 1995 (Italy)	Case-control: 746 cases and 2,053 hospital-based controls	29 items	Hospital recruitment, pathology confirmed	↑
	Pobel et al. 1995 (France)	Case-control: 92 cases and 128 hospital-based controls	61 items	Hospital recruitment, pathology confirmed	↑

Table 2-4. Overview of Epidemiological Studies of N-Nitrosodimethylamine Dietary Intake and Cancers

Cancer site	Citation (location)	Study type and population size	Food and beverage intake questionnaire	Case identification	Results[a]
Colon/rectum	Loh et al. 2011[b] (United Kingdom)	Cohort: 23,363 men and women; 276 colon and 137 rectal cancer cases; mean follow-up 11.4 years	131 items; administered at baseline	Cancer registry	↑ for rectum; ↔ for colon
	Knekt et al. 1999 (Finland)	Cohort: 9,985 men and women; 73 cases; maximum follow-up 24 years	Number of items not reported; administered at baseline	Cancer registry	↑
	Zhu et al. 2014 (Canada)	Case-control: 1,760 cases and 2,481 population-based controls	170 items	Regional familial cancer registries, pathology confirmed	↑
Esophagus	Keszei et al. 2013 (Netherlands)	Case-control: 151 cases of esophageal squamous cell carcinoma, 151 cases of esophageal adenocarcinoma and 4,032 control men and women; mean follow-up 14.3 years	150 items, administered at baseline	Cancer registry, pathology confirmed	↑ for squamous cell carcinoma, ↔ for adenocarcinoma
	Loh et al. 2011[b] (United Kingdom)	Cohort: 23,363 men and women; 55 cases; mean follow-up 11.4 years	131 items; administered at baseline	Cancer registry	↔
Upper aero-digestive tract	Rogers et al. 1995 (United States/ Washington state)	Case-control: 645 cases and 45 population-based controls	125 items	Cancer Surveillance System	↔
Head and neck	Knekt et al. 1999 (Finland)	Cohort: 9,985 men and women; 48 cases; maximum follow-up 24 years	Number of items not reported; administered at baseline	Cancer registry	↔
Liver	Zheng et al. 2021 (United States/Texas)	Case-control: 827 cases and 1,013 controls	131 items	Hospital recruitment, histologically or radiologically confirmed	↑ for plant sources of NDMA ↔ for animal sources of NDMA or all sources

Table 2-4. Overview of Epidemiological Studies of N-Nitrosodimethylamine Dietary Intake and Cancers

Cancer site	Citation (location)	Study type and population size	Food and beverage intake questionnaire	Case identification	Results[a]
Brain/spinal cord (glioma/ meningioma)	Michaud et al. 2009 (United States)	Cohorts: 49,935 men (HPFS), 92,468 women (NHS I), 95,391 women (NHS II); 133 cases of glioma among men and 202 among women; maximum follow-up 18 years (HPFS), 24 years (NHS I), and 14 years (NHS II)	61 or 130 items; administered at baseline and every 4 years	Self-identification at biennial questionnaire, confirmed by review of medical and pathology records	↔ in individual or pooled cohorts
	Giles et al. 1994 (Australia)	Case-control: 409 glioma cases and 409 population-based controls	59 items	Hospital recruitment	↑ among men
	Boeing et al. 1993 (Germany)	Case-control: 115 glioma and 81 meningioma cases and 418 population-based controls	42 items	Clinic recruitment, pathology confirmed	↑
Bladder	Jakszyn et al. 2011 (Europe)	Cohort: 481,419 men and women; 1,001 cases; mean follow-up 8.7 years	Number of items not reported; administered at baseline	Cancer registries, health insurance records, cancer and pathology hospital registries, and active follow-up	↔
Pancreas	Zheng et al. 2019 (United States/Texas)	Case-control: 1,110 cases and 1,010 controls recruited among friends, spouses, and in-laws of patients with other cancer types	84 or 131 items; administered at baseline	Hospital recruitment, pathology confirmed	↑

Table 2-4. Overview of Epidemiological Studies of N-Nitrosodimethylamine Dietary Intake and Cancers

Cancer site	Citation (location)	Study type and population size	Food and beverage intake questionnaire	Case identification	Results[a]
Lung	Loh et al. 2011[b] (United Kingdom)	Cohort: 23,363 men and women; 235 cases; mean follow-up 11.4 years	131 items; administered at baseline	Cancer registry	↔
	De Stefani et al. 1996 (Uruguay)	Case-control: 320 cases and 320 hospital-based controls	70 items	Hospital recruitment	↑

[a]↑: significant association (confidence limits for the effect estimate do not include 1.0); ↔: no significant association (confidence limits for the effect estimate include 1.0).
[b]Loh et al. (2011) observed no association between N-nitrosodimethylamine intake and breast, prostate, or ovarian cancers.

HPFS = Health Professionals Follow-Up Study; NHS = Nurses' Health Study

bias in the studies used in the NDMA. Geographic area was identified as a primary source of heterogeneity, but this factor was not the only source. In sensitivity analyses, the small case-control study by De Stefani et al. (1998) was shown to influence the association; without this study, the relative risk was 1.18 (95% CI 0.97–1.43).

Studies of dietary intake of NDMA and other cancer types are more limited. As shown in Table 2-4, two cohort studies and one case control study (Knekt et al. 1999; Loh et al. 2011; Zhu et al. 2014) observed positive associations between NDMA exposure and cancers of the colon or rectum. Findings are inconclusive for associations between NDMA and esophagus (Keszei et al. 2013; Loh et al. 2011) and lung cancers (De Stefani et al. 1996; Loh et al. 2011). In a case-control study of hepatocellular carcinomas, an association was observed with NDMA from plant-based sources (primarily grains and rice), but not with animal-based food sources or when both sources were combined (Zheng et al. 2021). Two older case-control studies (Boeing et al. 1993; Giles et al. 1994) reported positive associations between NDMA intake and gliomas and/or meningiomas.

However, analysis of three large cohorts (the Health Professionals Study and Nurses' Health Studies I and II) with follow up of at least 14 years showed no association with glioma, either in individual cohorts or pooled analysis (Michaud et al. 2009). Single studies of dietary NDMA and several other cancer types were evaluated. Positive associations were observed for the upper aerodigestive tract (Rogers et al. 1995) and pancreas (Zheng et al. 2019), but not for cancers of the head and neck (Knekt et al. 1999), bladder (Jakszyn et al. 2011), or breast, prostate, or ovaries (Loh et al. 2011).

Animal Studies. Three animal studies showed some evidence for induction of cancers after inhalation exposure. Klein et al. (1989, 1991) observed increases in the incidences of nasal tumors (aesthioneuroblastomas, mucoepidermoid tumors, squamous cell carcinomas, and neurogenic and osteogenic sarcomas) at all exposure levels (0.04, 0.2, and 1 ppm) in female rats exposed by inhalation to NDMA 4 hours/day, 5 days/week for ~72 weeks and observed until death. The incidences of nasal tumors were not clearly or consistently reported in the two publications but were increased with exposure based on available information indicating that zero or one control developed a nasal tumor. Twice weekly 30-minute exposures to 50 or 100 ppm NDMA vapor for life produced malignant nasal cavity tumors in rats (Druckrey et al. 1967). The incidence of tumors was 67% in each group, and the time to induce tumors in 50% of the rats was 400 days. Group sizes were small (12 and 6 animals at 50 and 100 ppm, respectively), control data were not reported, and additional information regarding longevity was not provided. Rats and mice that were continuously exposed to 0.07 ppm NDMA for 25 and

17 months, respectively, developed significantly increased incidences of lung, liver, and kidney tumors (Moiseev and Benemanski 1975). Tumor types included various adenomas, carcinomas, and sarcomas in the lung, liver and kidneys, and hemangiomas in the liver, but the types were not tabulated according to species or concentration. Induction of nasal tumors was not reported. Exposure to 0.002 ppm NDMA according to the same schedule did not produce significantly increased incidences of tumors in either species.

The carcinogenicity of orally administered NDMA has been demonstrated unequivocally in acute-, intermediate-, and chronic-duration studies with rats, mice, hamsters, and mink. The liver and lungs are the primary targets for NDMA carcinogenesis but tumors of the kidneys and testes can also occur. Incidences of liver and lung tumors are generally very high (often 50–100%), but liver tumors appear to occur most frequently in rats and hamsters and lung tumors appear to occur most frequently in mice. The liver tumors are usually hemangiosarcomas and hepatocellular carcinomas, and lung tumors are usually adenomas and liver tumor metastases.

A single dose of 5 mg/kg NDMA administered by gavage resulted in a significantly increased incidence of lung tumors (15/30 versus 4/30 in controls) in A/JNCr mice when sacrificed 16 weeks after dosing; no significant increase was seen with a single dose of 1 mg/kg (Anderson et al. 1992a). Daily treatment of Swiss mice with 10 mg/kg/day in drinking water for 1 week produced kidney and lung tumors (Terracini et al. 1966). Incidences of kidney and lung adenomas were 6/10 and 10/10 females, respectively. There were no kidney tumors in controls; lung adenoma incidences in controls were 2/17 females and 2/5 females (Terracini et al. 1966). Low incidences of epithelial tumors (8.6%) and mesenchymal tumors (14.5%) developed in the kidneys of rats following treatment with 8 mg/kg/day for 6 days (Ireton et al. 1972; McGiven and Ireton 1972). Evaluation of the results of McGiven and Ireton (1972) and Ireton et al. (1972) is complicated by the lack of a control group.

Aleksandrov (1974) reported possible evidence of transplacental carcinogenesis in rats exposed once to NDMA. A single dose of 30 mg/kg was administered by gavage to pregnant rats on GD 21, and offspring were necropsied at the time of natural death (~274 days after exposure). Histological examination of the offspring showed tumors (sites not reported) in 5 of 20 animals. Confidence in this finding is low, however, as the study did not report control findings or specific tumor types.

Numerous oral carcinogenicity studies of NDMA of intermediate duration (with exposure durations between 20 and 40 weeks) have been conducted in rats, mice, and hamsters. Carcinogenicity (liver, lung,

and/or kidney tumors) was observed in all studies. For example, rats administered NDMA in the drinking water at doses ≥0.3 mg/kg/day for 30 weeks developed malignant liver tumors (Keefer et al. 1973; Lijinsky and Reuber 1984; Takahashi et al. 2000). Lijinsky et al. (1987) observed high incidences of liver, lung, and kidney tumors in rats that were treated by gavage with 6 mg/kg twice weekly for 30 weeks. Untreated or vehicle controls were not used in the latter study, which compared tumorigenicity of different nitrosamines. However, similar findings were observed in a subsequent study with an untreated control (Lijinsky and Kovatch 1989), in which liver and kidney tumors were observed at NDMA doses ≥8.1 mg/kg (by gavage) twice weekly for 20–30 weeks. In a diet study with rats (10/sex exposed), daily treatment with a dose of 3.9 mg/kg for 26–40 weeks resulted in hepatic tumors in 19/20 animals (Magee and Barnes 1956). Neither Lijinsky and Kovatch (1989) nor Magee and Barnes (1956) reported the control incidences of tumors.

NDMA is also a carcinogen in mice. Liver, lung, and/or kidney tumors developed in mice after daily exposure to NDMA via drinking water at doses of ~1 mg/kg/day for 4, 13, 16, or 38 weeks (Anderson 1988; Anderson et al. 1992a; Den Engelse et al. 1974; Terracini et al. 1966), 1.8 mg/kg/day for 7 weeks (Clapp and Toya 1970), 0.91 for 38 weeks (Clapp and Toya 1970), ~0.4 mg/kg/day for 32 or 58 weeks (Clapp and Toya 1970), or 0.25 mg/kg/day for 32–48 weeks (Anderson et al. 1992a). In studies where NDMA was administered via the diet, doses of 13 mg/kg for 16–92 days (Otsuka and Kuwahara 1971), 5.26 mg/kg for 5 months (Takayama and Oota 1965), or 9.04 mg/kg for 10 months (Takayama and Oota 1965) also induced liver, lung, and/or kidney tumors in mice. Confidence in the results from Otsuka and Kuwahara (1971) and the 10-month experiment reported by Takayama and Oota (1965) is low, as both lacked appropriate control groups. In the only intermediate-duration gavage study with mice, twice weekly doses of 1 mg/kg for 50 weeks resulted in high (37–53%) incidences of malignant liver tumors (Griciute et al. 1981).

Intermediate-duration oral studies in hamsters and mink provided supporting evidence for NDMA carcinogenicity but were hampered by the lack of control or other study quality limitations. Daily administration of 4 mg/kg/day in the drinking water to hamsters for 12 or 16 weeks resulted in high incidences of cholangiocellular adenocarcinomas (Ungar 1986). Hamsters that were treated with NDMA by gavage twice weekly with a dose of 5.4 mg/kg for 6.5 weeks, once weekly with a dose of 10.7 mg/kg for 4 weeks, once weekly with a dose of 5.4 mg/kg for 20 weeks, or via drinking water with a dose of 1.1 mg/kg/day for 7 months developed high (60–79%) incidences of liver tumors (Bosan et al. 1987; Lijinsky et al. 1987). However, control groups were not included in the studies by Lijinsky et al. (1987) and Bosan et al. (1987). Hemangiomatous liver tumors occurred in 55% of deceased mink that received

NDMA in the diet at an estimated dose of 0.18 mg/kg/day (Martino et al. 1988); limitations of this study include uncertainty regarding exposure duration and concentration, examination only of animals that died, and use of historical controls.

Chronic oral carcinogenicity studies of NDMA have been conducted with rats, mice, and mink; these studies showed dose-related increases in the incidences of liver and testicular tumors in rats, liver and lung tumors in mice, and liver tumors in mink.

The largest and most comprehensive carcinogenicity study of NDMA was conducted by Peto et al. (1984, 1991a, 1991b). Groups of 60 rats/sex were exposed to 1 of 15 concentrations of NDMA in drinking water (between 0.033 and 16.896 ppm), yielding estimated doses of 0.001–0.697 mg/kg/day (Peto et al. 1991b). Controls received untreated water. Groups of six rats/sex/dose were sacrificed after 12 and 18 months; however, data from the interim sacrifices were not reported separately. The remaining animals were observed until natural death, moribund appearance, or appearance of palpable liver abnormalities (up to 3.5 years). Histopathology examinations were performed on grossly observed lesions; apart from these, "a few" sections of apparently normal liver and esophagus were routinely examined microscopically. In both male and female rats, NDMA doses ≥0.022 mg/kg/day (0.528 ppm) were associated with decreased survival due to liver tumors. The liver tumors included malignant hepatocellular, mesenchymal, and Kupffer cell tumors as well as benign tumors of the bile ducts. The incidences were reported separately for fatal and incidental tumors; most tumors were fatal. The incidences of any liver tumor (summed across cell type and fatal/incidental) were statistically significantly increased at doses ≥0.022 mg/kg/day. In analyses pooled across male and female rats, statistically significant trends for dose-related increases in the incidences of tumors (malignant or benign) at other sites (presumably detected at gross necropsy, as histopathology evaluations were not routinely performed for other organs) were reported for the prostate, seminal vesicles, or Cowper's complex; bronchus or lung; skin; and lymphatic or hematopoietic tissues.

Increased incidences of liver tumors occurred in Wistar rats that received ≥0.14 mg/kg/day doses of NDMA in the diet for 96 weeks; no increase was seen at 0.013 mg/kg/day (Arai et al. 1979; Ito et al. 1982). At the highest dose, female rats also exhibited a significantly increased incidence of leukemia (not further characterized; Arai et al. 1979). In a preliminary report that suffered from limitations in reporting (study design, implementation, and findings), Crampton (1980) administered NDMA to rats in the drinking water at concentrations between 0.033 and 1.69 ppm doses for life and reported increased liver tumor incidences at 0.132 ppm. The study authors estimated a dose range of 0.002–1.5 mg/kg/day;

however, the dose range (750-fold) appears to be inconsistent with the concentration range (51-fold), and efforts to validate the dose estimates using standard methodologies were not successful; thus, reliable dose estimates cannot be determined for this study. Two studies reported increased incidences of testicular tumors in rats exposed to NDMA. Terao et al. (1978) observed an increase in the incidence of testicular Leydig-cell tumors (7/15 versus 0/30 controls) in Wistar rats treated with 0.5 mg/kg/day of NDMA in the diet for 54 weeks. In contrast with other studies of rats exposed to doses in this range (e.g., Arai et al. 1979; Keefer et al. 1973; Lijinsky and Reuber 1984), these authors observed no tumors in the liver or other tissues. Nonsignificant increases in the incidences of testicular tumors were reported in Wistar rats exposed to 0.13 and 1.3 mg/kg/day NDMA in feed (60 and 52.9% compared with 28.6% in controls; Arai et al. 1979)

In A/JNCr mice exposed to NDMA in drinking water at a dose of 0.24 mg/kg/day for 72 weeks, the average number of lung tumors per tumor-bearing mouse was significantly increased (2.4 versus 1.5 in controls) (Anderson et al. 1992a). The incidence of tumors in treated mice did not differ from controls (88 versus 83%); however, this strain of mouse has a high spontaneous incidence of lung tumors. Clapp and Toya (1970) administered NDMA to RF mice via drinking water at daily doses of 0.43 and 0.91 mg/kg/day for life and observed that incidences of lung tumors and liver hemangiosarcomas were significantly increased at both doses; mean survival time at the low and high doses were 12 and 17 months, respectively. Hemangiomatous liver tumors developed in mink exposed to 0.1 mg/kg/day NDMA in the diet for 321–607 days (Koppang and Rimeslatten 1976).

One intermediate-duration study of cancer in animals exposed by dermal application of NDMA was located. A low incidence of lung adenomas (13%), but no skin tumors, developed in hairless mice that were treated once weekly with 33.3 mg/kg topical doses of NDMA for 20 weeks (Iversen 1980). Lung and skin tumors were not observed in historical control groups. Although Iversen (1980) concluded that the lung cancers were related to the topical applications of NDMA, it should be noted that the mice were housed in groups of eight in each cage, so oral exposure via grooming cannot be ruled out. In addition, inhalation exposure was possible as the application site was not occluded.

Mechanisms. The World Health Organization (WHO 2008) reviewed the mechanisms of NDMA carcinogenicity. NDMA is believed to induce cancer via genotoxicity induced by reactive metabolites, especially the methyldiazonium ion (see Section 3.1.3 for further detail). This intermediate is an alkylating agent that methylates DNA, forming several adducts including N^7-methylguanine, O^6-methylguanine, N^3-methyladenin, and O^4-methylthymine. The predominant adducts are

N^7-methylguanine (65% of all adducts) and O^6-methylguanine (7%). Depurination of the N^7-methylguanine adduct results in apurinic sites that can, if unrepaired, result in mutations (G-T transversions). The O^6-methylguanine adduct, while not the predominant adduct seen after NDMA exposure, is persistent and its relationship to mutations (G:C to A:T transitions) leading to carcinogenicity is well-established. These transition mutations have been detected in lung tumors of mice exposed to NDMA and in transgenic mice exposed to NDMA (reviewed by WHO 2008).

Souliotis et al. (1995, 2002) conducted experiments to investigate the relationship between O^6-methylguanine adducts and liver tumors in rats, using drinking water concentrations at which Peto et al. (1984, 1991a, 1991b) observed liver tumors. Souliotis et al. (1995) observed that the kinetics of O^6-methylguanine adduct accumulation did not fully explain the increase in cancer incidence reported by Peto et al. (1984, 1991a, 1991b). Steady-state adduct accumulation exhibited a small decrease in slope at doses >0.056 mg/kg/day, in contrast to the sharp increase in liver tumor incidences above this dose. In a subsequent experiment in rats, Souliotis et al. (2002) demonstrated increased DNA replication in rat hepatocytes after exposure to NDMA at concentrations >1 ppm (~0.044 mg/kg/day in the study by Peto et al. 1984, 1991a, 1991b). The authors suggested that the hepatic carcinogenicity of NDMA in rats was influenced both by DNA damage and increased replication.

The O^6-methylguanine adduct can be repaired by O^6-methylguanine DNA-methyltransferase (MGMT), and indeed reduced expression of this enzyme is seen in many tumor types (Sharma et al. 2009). Nakatsuru et al. (1993) demonstrated that transgenic mice expressing higher levels of MGMT develop fewer tumors after NDMA exposure than those expressing normal levels. Expression and activity of MGMT vary across tissues and by age and species, and polymorphisms of the enzyme have also been identified (Sharma et al. 2009; WHO 2008). These variations may contribute to tissue, species, and population differences in adduct accumulation and tumor susceptibility. In humans, MGMT activity is highest in the liver, followed by lung, kidney, and colon, with lower levels in the pancreas, hematopoietic and lymphoid cells, and brain (Sharma et al. 2009). In patas monkeys exposed once to 0.1 mg/kg NDMA by gavage, the highest levels of O^6-methylguanine adducts were detected in the gastric mucosa and liver; levels in leukocytes, esophagus, ovary, pancreas, urinary bladder, and uterus were about half the levels in gastric mucosa and liver (Anderson et al. 1996). In the same study, MGMT activities were highest in the liver > stomach > pancreas ≈ colon ≈ kidney ≈ small intestine.

There is some evidence that MGMT activity may be higher in humans than in laboratory rodents. Gerson et al. (1986) measured MGMT activity *in vitro* in tissues from humans, rats, and mice. In the liver,

intestine, lungs, brain, lymphocytes, and bone marrow, the MGMT activity in humans was higher than in rats or mice. In contrast, both rats and mice had higher MGMT activity in the kidney than humans. The study authors noted that there was substantial variation in activity levels between individual human donors and between individual animals, suggesting that some individuals may have lower MGMT activity and thus be at higher risk from exposure to alkylating agents such as NDMA (Gerson et al. 1986).

Kay et al. (2021) showed the importance of the mammalian alkyladenine DNA glycosylase (AAG) enzyme, which removes methylated bases and is the first step in base excision repair, in determining the carcinogenic action of NDMA. Using mice with the *Aag* gene knocked out (resulting in increases in replication-blocking 3-methyl adenine adducts) as well as mice overexpressing *Aag* (resulting in increased DNA strand breaks), the study authors showed that the absence of the *Aag* gene increased NDMA-induced cancer incidence relative to wild-type mice (86 versus 67%, with 4.5 tumors/mouse versus 1 tumor/mouse, respectively). In contrast, the overexpression of the *Aag* gene reduced cancer incidence, but resulted in early mortality (13% within 2 weeks of exposure compared with 0.7% of wild-type mice) (Kay et al. 2021).

O^6-methylguanine adducts were detected in fetal tissues of patas monkeys exposed to NDMA during pregnancy (Chhabra et al. 1995). In addition, Anderson et al. (1989) reported significantly increased incidences of hepatocellular carcinomas in offspring of C3H/HeNCr MTV^- mice given 7.4 mg/kg NDMA by i.p. injection on GD 16 or 19. These studies provide support for the findings of Aleksandrov (1974), who reported tumors (sites unspecified) in the offspring of rats exposed orally to NDMA on GD 21.

As discussed in Section 2.20, both *in vitro* and *in vivo* tests for mutagenicity of NDMA have consistently shown positive results both with and without metabolic activation.

HHS concluded that NDMA is "reasonably anticipated to be a human carcinogen," based on sufficient evidence in animals (NTP 2021). EPA (IRIS 1987) classified NDMA in Group B2 (probable human carcinogen) based on sufficient evidence of carcinogenicity in animals. In addition, IARC (1987) assigned NDMA to Group 2A (probably carcinogenic to humans) based on inadequate information in humans and sufficient evidence in experimental animals. EPA's Integrated Risk Information System (IRIS) reports an oral slope factor of 51 per mg/kg/day and an inhalation unit risk of 0.014 per $\mu g/cm^3$ for NDMA. For its Six-Year Review 3 Technical Support Document for Nitrosamines (EPA 2016), EPA's Office of Water derived an oral slope factor of 21 per mg/kg/day for NDMA using the Peto et al. (1991a,

1991b) study, which had not been published at the time when EPA's IRIS review of NDMA was prepared (1987).

2.20 GENOTOXICITY

Methylated DNA adducts (7-methylguanine and O^6-methylguanine) were detected in the liver of a 23-year-old man who died from suspected NDMA poisoning (Herron and Shank 1980). No other studies of genotoxicity in humans exposed to NDMA were located. NDMA has been extensively tested for genotoxicity in both *in vitro* and *in vivo* animal systems, yielding positive results in most assays. As a result, NDMA is routinely used as a positive control in genotoxicity studies.

Table 2-5 provides an overview of the *in vitro* results; the studies presented are representative of the database, but do not reflect every available study. *In vitro* assays have demonstrated increased mutation frequencies in bacteria, yeast, and mammalian cell systems incubated with NDMA with metabolic activation (see Table 2-5). Increases in the frequency of chromosomal aberrations have been observed in several rat cell types, Chinese hamster lung, ovary, and fibroblast cells, and in human fibroblast cells. As with the mutation assays, the positive results were seen in the presence of exogenous metabolic activation or in metabolically competent cell systems. *In vitro* tests for micronuclei have shown mixed results; increases in micronuclei were observed in human lymphoblastoid cells (Crofton-Sleigh et al. 1993) and in human hepatoma (HepG2) cells (Valentin-Severin et al. 2003) tested without metabolic activation, and in Chinese hamster lung cells tested with activation (Matsushima et al. 1999). Assays with other human cell types and with rat and mouse cells yielded negative results (see Table 2-5). In a large number of other *in vitro* tests, NDMA was shown to induce sister chromatid exchanges and DNA damage, repair synthesis, or unscheduled synthesis (see Table 2-5).

Table 2-5. Genotoxicity of N-Nitrosodimethylamine *In Vitro*

		Results		
		Activation		
Species (test system)	Endpoint	With	Without	Reference
Salmonella typhimurium	Gene mutation	+	NT or –	Araki et al. 1984; Bartsch et al. 1980; Bringezu and Simon 2022; De Flora et al. 1984; Ishidate and Yoshikawa 1980; Langenbach et al. 1986; Prival and Mitchell 1981; Surh et al. 1995; Wagner et al. 2014; Wang et al. 2017

Table 2-5. Genotoxicity of N-Nitrosodimethylamine *In Vitro*

Species (test system)	Endpoint	Results		Reference
		Activation		
		With	Without	
Escherichia coli	Gene mutation	+	NT	Araki et al. 1984; Bringezu and Simon 2022; De Flora et al. 1984; Jiao et al. 1993
Saccharomyces cerevisae	Gene mutation	+	NT	Frezza et al. 1983; Jagannath et al. 1981
Human lymphoblastoid (AHH-1, MCL-5, MCL-1) cells	Gene mutation	NA	+	Davies et al. 1989; Dobo et al. 1997, 1998
Chinese hamster V79 and ovary cells	Gene mutation	+	–	Adair and Carver 1983; Bartsch et al. 1980; Carver et al. 1981; Dickins et al. 1985; Hsie et al. 1978; Katoh et al. 1982; Kuroki et al. 1977; Langenbach 1986; Lawson and Kolar 1992; O'Neill et al. 1982; Swedmark et al. 1994
Mouse lymphoma L578Y cells	Gene mutation	+	–	Amacher and Paillet 1983; Clive et al. 1979
Rat hepatocyte (NRL cl-B, NRL cl-C, and ARL) cell lines	Chromosomal aberrations	NT	+	Kulka et al. 1993
Chinese hamster lung, ovary, or V79 fibroblast cells	Chromosomal aberrations	+	NT or –	Bean et al. 1994; Ishidate and Yoshikawa 1980; Johnson et al. 1996; Kulka et al. 1993; Matsuoka et al. 1979, 1986; Matsushima et al. 1999
Human fibroblast (L136) cells	Chromosomal aberrations	+	NT	Bean et al. 1994
Rat ascites hepatoma (AH66B) and rat esophageal (R1, R3) tumor cells	Chromosomal aberrations	NT	+	Ikeuchi and Sasaki 1981
Human lymphoblastoid (MCL5) cells	Micronuclei	NT	+	Crofton-Sleigh et al. 1993
Human peripheral blood lymphocytes	Micronuclei	–	NT	Katic et al. 2010
Human lymphoblasts (TK6) and peripheral blood lymphocytes	Micronuclei	NT	–	Liviac et al. 2011
Human hepatoma (HepG2) cells	Micronuclei	NT	+	Valentin-Severin et al. 2003
Rat hepatoma (H4IIEC3) cells	Micronuclei	NT	–	Roscher and Wiebel 1989
Mouse embryo fibroblast (NIH3T3) cells	Micronuclei	NA	–	Wang et al. 2017
Chinese hamster lung cells	Micronuclei	+	NT	Matsushima et al. 1999

Table 2-5. Genotoxicity of N-Nitrosodimethylamine *In Vitro*

		Results		
		Activation		
Species (test system)	Endpoint	With	Without	Reference
Primary rat hepatocytes	Sister chromatid exchange	NT	+	Eckl et al. 1987
Rat hepatocyte (NRL cl-B, NRL cl-C, and ARL) cell lines	Sister chromatid exchange	NT	+	Kulka et al. 1993
Rat esophageal tumor, ascites hepatoma	Sister chromatid exchanges	NT	+	Abe and Sasaki 1982; Ikeuchi and Sasaki 1981
Human lymphocytes	Sister chromatid exchange	+	–	Inoue et al. 1983; Madle et al. 1987
Human fibroblasts	Sister chromatid exchange	+	NT	Tomkins et at. 1982
Chinese hamster ovary cells	Sister chromatid exchange	+	NT	Blazak et al. 1985; Johnson et al. 1996; Okinaka et al. 1981; Tomkins et al. 1982
Chinese hamster V79 fibroblast cells	Sister chromatid exchange	+	–	Blazak et al. 1985; Kulka et al. 1993; Madle et al. 1987; Sirianni and Huang 1987
Chinese hamster primary lung cells	Sister chromatid exchange	+	–	Shimizu et al. 1984
Rat hepatocytes	DNA damage	NT	+	Bermudez et al. 1982; Bradley et al. 1982; Martelli et al. 1988; Pool et al. 1988; Singh and Roscher 1991
Rat hepatoma (H4IIEC3) cells	DNA damage	NT	+	Singh and Roscher 1991
Human hepatocytes	DNA damage	NT	+	Martelli et al. 1985, 1988
Human hepatoma (HepG2, HepaRG) cells	DNA damage	NT	+	Erkekoglu and Baydar 2010; Le Hegarat et al. 2010; Uhl et al. 1999; Valentin-Severin et al. 2003
Human lung or kidney cells	DNA damage	NT	+	Robbiano et al. 2006
Rat lung or kidney cells	DNA damage	NT	+	Robbiano et al. 2006
Rat kidney cells	DNA damage	NT	–	Brendler et al. 1992
Human lymphoblasts (TK6)	DNA damage	–	+	Liviac et al. 2011
Human hepatoma (HepG2) cells	DNA damage	NT	+	Valentin-Severin et al. 2003
Chinese hamster ovary cells	DNA damage	+	–	Wagner et al. 2014
Mouse splenocytes	DNA damage	+	–	Kim et al. 1989
Mouse embryo fibroblast (NIH3T3) cells	DNA damage	NA	–	Wang et al. 2017

Table 2-5. Genotoxicity of N-Nitrosodimethylamine *In Vitro*

Species (test system)	Endpoint	Results		Reference
		Activation		
		With	Without	
Rat hepatocytes	DNA methylation/ adducts	NT	+	Lachapelle et al. 1994; Lachapelle et al. 1992
S. cerevisae	DNA repair	NT	+	He et al. 2021
Rat hepatocytes	DNA repair synthesis	NT	+	Andrae and Schwarz 1981; Rossberger et al. 1987
Rat hepatoma (H4IIEC3) cells	DNA repair synthesis	NT	+	Rossberger et al. 1987
Human hepatocytes	DNA repair synthesis	NT	+	Martelli et al. 1988
Rat hepatocytes	Unscheduled DNA synthesis	NT	+	Martelli et al. 1988; Shaddock et al. 1993
Human lymphoblasts	Unscheduled DNA synthesis	+	NT	Andrae et al. 1979
Mouse hepatocytes	Unscheduled DNA synthesis	NT	+	McQueen et al. 1983
Hamster hepatocytes	Unscheduled DNA synthesis	NT	+	McQueen et al. 1983
Rat pancreatic cells	Unscheduled DNA synthesis	NT	–	Steinmetz and Mirsalis 1984

+ = positive results; – = negative results; DNA = deoxyribonucleic acid; NT = not tested

Table 2-6 provides an overview of the *in vivo* results; the studies presented are representative of the database, but do not reflect every available study. NDMA has been tested extensively for mutagenicity in transgenic rodent models including the Big Blue® and Big Blue® cII rat and Big Blue®, Big Blue® cII, and Muta™ mouse (reviewed by Lambert et al. 2005; see also Table 2-6). In these studies, NDMA was administered orally (diet or gavage) or via i.p. injection for one or more days at doses between 1.8 and 54 mg/kg/day. Tissues, including liver, lung, kidney, bone marrow, spleen, bladder, and forestomach were sampled for mutations from 1 to 183 days after exposure. In these experiments, NDMA has consistently yielded increased mutations in the liver regardless of species, exposure route, duration, sampling time, and transgene (*lacI, cII, lacZ*). In mice, increased mutation frequencies were also observed in the lung and kidney (reviewed by Lambert et al. 2005).

Table 2-6. Genotoxicity of N-Nitrosodimethylamine *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Rat kidney	Mutations	+	Horesovsky et al. 1995
Rat (transgenic Big Blue® and Big Blue® cII) liver	Mutations	+	Gollapudi et al. 1998
Mouse (transgenic Big Blue®) liver, lung, kidney	Mutations	+	Ashby et al. 1994; Butterworth et al. 1998; Cunningham et al. 1996; Davies et al. 2000; Delker et al. 2008; Hayward et al. 1995; Lefevre et al. 1994; Mirsalis et al. 1993; Shane et al. 1999, 2000a, 2000b; Shephard et al. 1995; Suzuki et al. 1996; Tinwell et al. 1994a, 1995
Mouse (transgenic Big Blue® cII) liver	Mutations	+	Shane et al. 2000b
Mouse (Muta™ mouse transgenic) liver, lung, spleen		+	Fletcher et al. 1998; Jiao et al. 1997; Lefevre et al. 1994; Souliotis et al. 1998; Suzuki et al. 1998; Tinwell et al. 1994b, 1995, 1998
Mouse (transgenic Big Blue®) testes, bone marrow, bladder, forestomach	Mutations	–	Ashby et al. 1994; Shephard et al. 1995; Suzuki et al. 1996
Mouse (Muta™ mouse transgenic) bone marrow, kidney	Mutations	–	Jiao et al. 1997; Souliotis et al. 1998; Suzuki et al. 1998
Mouse intestine	Mutations	+	Winton et al. 1990
Mouse lymphocytes	Mutations	–	Dass et al. 1998
Mouse lung tumors	Mutations	+	Chen et al. 1994; Devereux et al. 1991; Ramakrishna et al. 2000
Drosophila melanogaster	Mutations	+	Blount et al. 1985; Brodberg et al. 1987; Goto et al. 1999; Koike et al. 2018; Lee et al. 1983; Negishi et al. 1991, 2020; Nivard et al. 1996; Vogel et al. 1990
Fish liver	Mutations	+	Hobbie et al. 2012
Rat liver	Aneuploidy	+	Clawson et al. 1992
Drosophila melanogaster	Aneuploidy	+	Woodruff and Seeger 1991
Hamster embryonic fibroblasts (transplacental)	Chromosome aberrations	+	Inui et al. 1979
Rat liver	Chromosome aberrations	+	Asakura et al. 1998; Sawada et al. 1991
Rat and mouse liver	Micronuclei	+	Braithwaite and Ashby 1988; Cliet et al. 1989; Hamada et al. 2015; Mehta et al. 1987; Sawada et al. 1991; Suzuki et al. 2005, 2009; Takashima et al. 2015; Tates et al. 1980
Rat kidney	Micronuclei	+	Robbiano et al. 1997
Rat bone marrow and spleen	Micronuclei	+	Krishna and Theiss 1995
Rat bone marrow	Micronuclei	+/–	Trzos et al. 1978

Table 2-6. Genotoxicity of N-Nitrosodimethylamine *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Rat bone marrow	Micronuclei	–	Hamada et al. 2015; Takashima et al. 2015
Mouse bone marrow and/or spleen	Micronuclei	+	Bauknecht et al. 1977; Fritzenschaf et al. 1993; Krishna et al. 1990; Morrison and Ashby 1994; Odagiri et al. 1986; Sato et al. 1992; Wild 1978
Mouse bone marrow	Micronuclei	–	Cliet et al. 1989, 1993
Rat peripheral blood	Micronuclei	–	Rothfuss et al. 2010
Mouse peripheral blood	Micronuclei	+	Sasaki 1991; Sato et al. 1992
Rat and mouse peripheral blood	Micronuclei	–	Suzuki et al. 1996, 2005
Rat stomach and colon	Micronuclei	–	Hamada et al. 2015; Takashima et al. 2015
Hamster embryonic fibroblasts (transplacental)	Micronuclei	+	Inui et al. 1979
Mouse spermatid	Micronuclei	+	Cliet et al. 1993
Hen egg	Micronuclei	+	Wolf et al. 2003
Rat liver	Sister chromatid exchanges	+	Sawada et al. 1991
Hamster bone marrow	Sister chromatid exchanges	+/–	Neal and Probst 1983
Mouse bone marrow	Sister chromatid exchanges	+	Bauknecht et al. 1977; Sharma et al. 1983
Drosophila melanogaster	Miotic recombination	+	Rodriguez-Arnaiz et al. 1996
Human liver	DNA methylation/ adducts	+	Herron and Shank 1980
Rat, mouse, hamster and/or gerbil liver	DNA methylation/ adducts	+	Bamborschke et al. 1983; Bianchini and Wild 1994; Camus et al. 1990; Chin et al. 1993; Dai et al. 1991; Fadlallah et al. 1994; Fan et al. 1989; Klaude et al. 1989; Kroeger-Koepke et al. 1992; Ma et al. 2015; O'Connor et al. 1982; Pegg and Hui 1978; Pegg et al. 1981; Scherer et al. 1989; Souliotis et al. 1995; Stumpf et al. 1979; Takahashi et al. 1996
Rat kidney, mammary glands, and leukocytes	DNA methylation/ adducts	+	Bianchini and Wild 1994; Chhabra et al. 2000; Fadlallah et al. 1994; Fan et al. 1989; Souliotis et al. 1995
Rat fetal liver, lung, and/or kidney	DNA methylation/ adducts	+	Chhabra et al. 2000
Rat esophagus	DNA methylation/ adducts	–	Scherer et al. 1989
Human placenta	DNA methylation/ adducts	–	Annola et al. 2009

Table 2-6. Genotoxicity of N-Nitrosodimethylamine *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Rat liver, lung, kidney, nasal cavity, and/or peripheral blood lymphocytes	DNA damage	+	Abanobi et al. 1979; Bermudez et al. 1982; Brambilla et al. 1981, 1987, 1992; Dahlhaus and Appel 1993; McNamee and Bellier 2015; Petzold and Swenberg 1978; Pool et al. 1990; Pool-Zobel et al. 1992; Rothfuss et al. 2010; Webster et al. 1996
Rat liver and kidney	DNA damage	+	Barbin et al. 1983
Rat kidney	DNA damage (double-strand breaks)	–	McLaren et al. 1994
Rat lung	DNA damage	–	Barbin et al. 1983
Mouse liver, kidney, bladder	DNA damage	+	Cesarone et al. 1982; Tsuda et al. 2001
Hamster liver	DNA damage	+	Barbin et al. 1983
Hamster lung	DNA damage	–	Barbin et al. 1983
Rat stomach	DNA damage	–	McNamee and Bellier 2015; Ohsawa et al. 1993; Okabe et al. 2019
Mouse colon	DNA damage	–	Tsuda et al. 2001
Fetal mouse liver and lung	DNA damage	+	Bolognesi et al. 1988
Drosophila melanogaster	DNA damage	+	Negishi et al. 1991
Rat liver	Unscheduled DNA synthesis	+	Asakura et al. 1994; Bakke and Mirsalis 1984; Doolittle et al. 1984, 1987; Kornbrust and Dietz 1985; Mirsalis and Butterworth 1980; Mirsalis et al. 1989; Sawada et al. 1989, 1995
Mouse liver	Unscheduled DNA synthesis	+	Mirsalis et al. 1989
Rat upper respiratory tract	Unscheduled DNA synthesis	+	Doolittle et al. 1984
Rat stomach	Unscheduled DNA synthesis	–	Ohsawa et al. 1993
Rat spermatocytes	Unscheduled DNA synthesis	–	Doolittle et al. 1984
Mouse testes	Unscheduled DNA synthesis	+	Cesarone et al. 1979
Rat embryo	Unscheduled DNA synthesis	+	Huang and Catalano 1994
Rat liver	Replicative DNA synthesis	+	Asakura et al. 1998
Mouse testes	Inhibition of DNA synthesis	+	Friedman and Staub 1976

– = negative result; + = positive result; +/– = equivocal results; DNA = deoxyribonucleic acid

Studies that examined the spectrum of mutations induced by NDMA have shown that the most common mutations in the Muta™ (*lacZ*) and Big Blue® (*lacI*) mouse are GC→AT transitions, primarily at non-CpG sites (Delker et al. 2008; reviewed by Lambert et al. 2005). GC→AT transitions can be produced if O^6-methylguanine adducts are not repaired, and this particular type of mutation in non-CpG sites is associated with an increased risk of cancer. Other mutations shown in these analyses included A:T→T:A transversions as well as single and multiple base pair deletions and frameshift mutations (Delker et al. 2008; reviewed by Lambert et al. 2005).

There is some evidence that younger animals may be more susceptible to NDMA mutagenicity. In one study, NDMA administration increased the mutation frequency in the livers of Big Blue (lacI) mice when administered as five daily doses of 2 mg/kg/day beginning at 3 weeks of age, but not when administered under the same conditions beginning at 6 weeks of age (reviewed by Lambert et al. 2005). The authors suggested that the difference in response could stem from age-related differences in metabolic activation, DNA adduct removal rates, or rates of mutation fixation. Delker et al. (2008) treated this same strain with three daily doses of 7 mg/kg/day beginning at 12 weeks of age and observed a significant increase in mutation frequency in the liver.

Along with the results in transgenic rodents, other *in vivo* studies have provided additional evidence for the genotoxicity of NDMA. As shown in Table 2-6, exposure to NDMA has resulted in mutations in rat kidney, mouse intestine and lymphocytes, *Drosophila melanogaster*, and fish liver; chromosomal aberrations or aneuploidy in rat liver, hamster fibroblasts, and *Drosophila*; and micronuclei in several species and tissues. In addition, NDMA has induced DNA methylation and adducts, DNA damage, and unscheduled DNA synthesis, especially in the liver, in a number of species (see Table 2-6).

Further discussion of the genotoxic mechanisms of cancers induced by NDMA, including specific DNA adducts, DNA repair enzymes, and tissue distribution of adducts and repair enzymes is presented in Section 2.19 (Cancer) under *Mechanisms*.

Taken together, the *in vitro* and *in vivo* genotoxicity data demonstrate unequivocally that one or more metabolites of NDMA is genotoxic to the liver in a wide range of species. In accordance with this finding, the liver is the primary target of NDMA carcinogenesis, suggesting that genotoxicity plays a role in the mechanism by which NDMA induces cancer.

NDMA has not shown genotoxic activity in germ cells *in vivo* or *in vitro*. No increase in unscheduled DNA synthesis was seen in spermatocytes of rats exposed to NDMA by inhalation (Doolittle et al. 1984). In addition, NDMA was negative for dominant lethal mutations in ICR/Ha Swiss mice exposed by i.p. injection (Epstein et al. 1972). In CF-1 mice exposed by i.p., intravenous (i.v.), or oral administration, ^{14}C NDMA did not alkylate sperm heads at doses from 4 to 14 mg/kg (Stott and Watanabe 1980). These study authors suggested that the lack of binding might stem from relatively low levels of the active NDMA metabolites in the testes resulting from low enzyme activity and short half-life of the metabolites. Despite the lack of germ cell genotoxicity in these studies, NDMA did induce O^6-methylguanine adducts in patas monkey fetuses (Chhabra et al. 1995), chromosomal aberrations and micronuclei in the embryos of treated pregnant hamsters (Inui et al. 1979), and transplacental carcinogenesis in mice exposed by i.p. injection (Anderson et al. 1989).