2. HEALTH EFFECTS

2.1 INTRODUCTION

This chapter contains descriptions and evaluations of studies and interpretation of data on the health effects associated with exposure to NDMA. Its purpose is to present levels of significant exposure for NDMA based on toxicological studies, epidemiological investigations, and environmental exposure data. This information is presented to provide public health officials, physicians, toxicologists, and other interested individuals and groups with (1) an overall perspective of the toxicology of NDMA and (2) a depiction of significant exposure levels associated with various adverse health effects.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the data in this section are organized first by route of exposure -- inhalation, oral, and dermal -- and then by health effect -- death, systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods -- acute, intermediate, and chronic.

Levels of significant exposure for each exposure route and duration (for which data exist) 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. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear, determine whether or not the intensity of the effects varies with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown on the tables and graphs may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons or with the identification of persons with the potential to develop such disease may be interested in levels of exposure associated with "serious" effects. Public health officials and project managers concerned with response actions at Superfund sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAEL) have been observed. Estimates of levels posing minimal risk to humans (minimal risk levels, MRLs) are of interest to health professionals and citizens alike.

For certain chemicals, levels of exposure associated with carcinogenic effects may be indicated in the figures. These levels reflect the actual

doses associated with the tumor incidences reported in the studies cited. Because cancer effects could occur at lower exposure levels, the figures also show estimated excess risks, ranging from a risk of one in 10,000 to one in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made, where data were believed reliable, for the most sensitive noncancer end point for each exposure duration. MRLs include adjustments to reflect human variability and, where appropriate, the uncertainty of extrapolating from laboratory animal data to humans. Although methods have been established to derive these levels (Barnes et al. 1987; EPA 1980a), uncertainties are associated with the techniques.

2.2.1 Inhalation Exposure

2.2.1.1 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 two weeks, and subsequently to an unknown level of fumes during cleanup of a spilled flask (Freund 1937). The subject became ill 6 days later, showed abdominal distention, large amounts of yellow ascitic fluid, a tender and enlarged liver and enlarged spleen, and 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).

The lethality of inhaled NDMA has been evaluated in several acute duration studies with animals. Four-hour single 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 predominant pathologic findings. Druckrey (1967) reported that the "LD_{50}" for rats exposed to NDMA by inhalation for one hour is 37 mg/kg. The air concentration corresponding to this dose is not reported but a value of 925 ppm can be calculated from information provided in the report; confidence in this value is low, however, 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 after 1-3 days. The 57 ppm mouse and 78 ppm rat LC_{50} values are presented in the acute duration category in Table 2-1 and Figure 2-1. The Druckrey (1967) rat value is not included in Table 2-1 and Figure 2-1 due to uncertainty regarding its validity. The 16 ppm concentration represents a LOAEL for lethality in dogs due to acute duration inhalation

TABLE 2-1. Levels of Significant Exposure to N-Nitrosodimethylamine - Inhalation

Graph Key	Species	Exposure Frequency/ Duration	Effect	NOAEL[b] (ppm)	LOAEL[a] (Effect) Less Serious (ppm)	LOAEL[a] (Effect) Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	rat	4 hr, once				78 (LC_{50})	Jacobson et al. 1955
2	mouse	4 hr, once				57 (LC_{50})	Jacobson et al. 1955
3	dog	4 hr, once				16 (death)	Jacobson et al. 1955
Systemic							
4	rat	4 hr, once	Hepatic			78 (hemorrhagic necrosis)	Jacobson et al. 1955
5	mouse	4 hr, once	Hepatic			57 (hemorrhagic necrosis)	Jacobson et al. 1955
6	dog	4 hr, once	Hemato			16 (increased clotting time)	Jacobson et al. 1055
7	dog	4 hr, once	Hepatic			16 (hemorrhagic necrosis)	Jacobson et al. 1955
CHRONIC EXPOSURE							
Cancer							
8	rat	life, 2 d/wk, 30 min/d				50 (CEL[c]) (nasal tumors)	Druckrey et al. 1967
9	rat	25 mo, continuous				0.07 (CEL) (liver, lung, kidney tumors)	Moiseev and Benemanski 1975
10	mouse	17 mo, continuous				0.07 (CEL) (liver, lung, kidney tumors)	Moiseev and Benemanski 1975

[a]NOAEL - No Observed Adverse Effect Level
[b]LOAEL - Lowest Observed Adverse Effect Level
[c]CEL - Cancer Effect Level

2. HEALTH EFFECTS

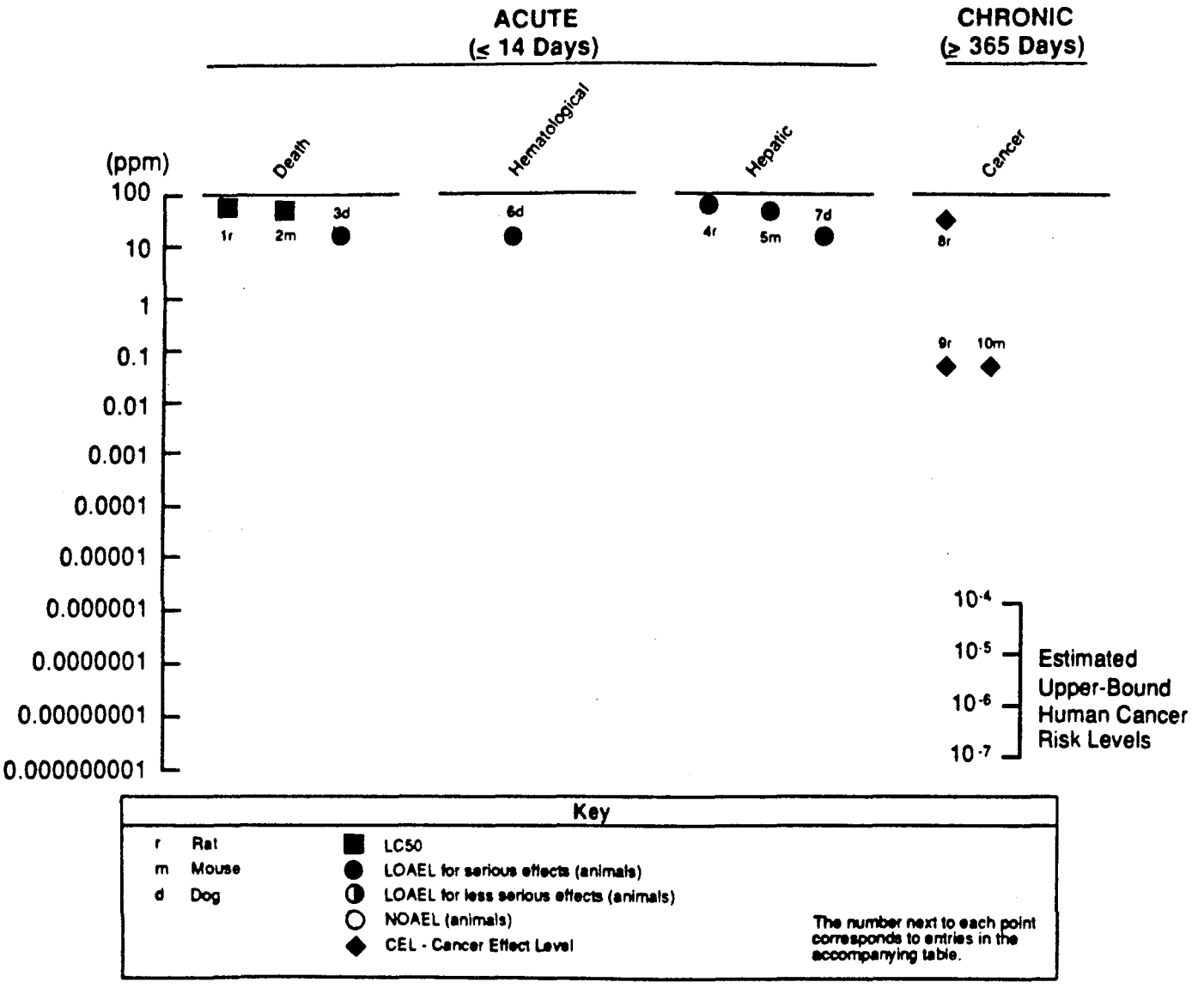

FIGURE 2-1. Levels of Significant Exposure to N-Nitrosodimethylamine - Inhalation

exposure (Table 2-l and Figure 2-l). The concentration of 16 ppm in air (Jacobson et al. 1955) is presented in Table l-2.

2.2.1.2 Systemic Effects

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

Respiratory Effects. Freund (1937) observed small hemorrhages in the bronchi and trachea of a person who died from accidental exposure to vapors of NDMA (Section 2.2.1.1).

No studies were located regarding respiratory effects in animals following.inhalation exposure to NDMA.

Cardiovascular Effects. Subpericardial hemorrhage was observed in a person who died from accidental exposure to vapors of NDMA (Freund 1937) (Section 2.2.1.1).

No studies were located regarding cardiovascular effects in animals following inhalation exposure to NDMA.

Gastrointestinal Effects. Gastrointestinal hemorrhage was observed in a person who died from accidental exposure to vapors of NDMA (Freund 1937) (Section 2.2.1.1).

No studies were located regarding gastrointestinal effects in animals following inhalation exposure to NDMA.

Hematological Effects. No studies were located regarding hematological effects in humans following inhalation exposure to NDMA.

Hematological determinations were performed in dogs that were exposed to 16-144 ppm NDMA for 4 hours (Jacobson et al. 1955). Increased coagulation time, increased prothrombin time, increased plasma cholinesterase levels and leukopenia occurred following exposure to all concentrations. There was no evidence of intravascular hemolysis. As indicated in Section 2.2.1.1, the concentrations producing these effects were lethal. Pathologic examination of the dogs showed bloody ascites and hemorrhage in the liver and other abdominal tissues, Due to the clinical evidence of impaired blood coagulation and the possibility that the hemorrhagic effects were related to impaired coagulation, 16 ppm is a LOAEL for hematological effects due to acute inhalation exposure (Table 2-l and Figure 2-l).

Hepatic Effects. 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 2.2.1.1. Of the subjects who did not die, 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 two 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).

Hepatotoxicity is a predominant effect of high concentrations of inhaled NDMA in animals. 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. As indicated in Section 2.2.1.1, the concentrations producing these effects were lethal. Jacobson et al. (1955) also indicated that necrosis and hemorrhage occurred in the liver of rats and mice that were exposed to lethal concentrations of NDMA for 4 hours; as indicated in Section 2.2.1.1, LC_{50} values for the rats and mice are 78 and 57 ppm, respectively. The 16 ppm, 57 and 78 ppm concentrations represent LOAELs for hepatic effects due to acute inhalation exposure and are presented in Table 2-l and Figure 2-l. The concentration of 16 ppm in air (Jacobson et al. 1955) is presented in Table l-2.

Dennal/Ocular Effects. No studies were located regarding dermal or ocular effects in humans following inhalation exposure to NDMA.

Limited information is available regarding dermal or ocular effects of inhaled NDMA. Doolittle et al. (1984) reported that the only toxic signs observed in rats exposed to 500 or 1000 ppm for 4 hours were reddened eyes and piloerection. The only additional information reported in this study pertained to genotoxic effects. Although high concentrations of NDMA vapor are likely to be irritating, the significance of the reddened eyes and piloerection cannot be determined because it is not specified if the effects occurred at both concentrations and prevalence is not indicated. As indicated in Section 2.2.1.1, acute exposure to much lower concentrations of NDMA was lethal for 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 consequently not observed for subsequent death.

No studies were located regarding the following effects in humans or animals following inhalation exposure to NDMA:

2.2.1.3 Immunological Effects

2.2.1.4 Neurological Effects

2.2.1.5 Developmental Effects

2.2.1.6 Reproductive Effects

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans following inhalation exposure to NDMA.

Rats exposed to 500 or 1000 ppm of NDMA in the air for 4 hours showed chemically induced DNA repair in epithelial cells in the nasal turbinates and trachea. DNA repair was also evident in hepatocytes, indicating that the substance entered the general circulation. No DNA repair was seen in the pachytene spermatocytes, indicating that NDMA either did not reach the testes in high enough concentrations, or that the testes could not metabolically activate the compound (Doolittle et al. 1984). It should be noted that the exposures in this study are likely to have been lethal if the rats had.been observed following treatment; as indicated in Section 2.2.1.1, 4-hour exposure to much lower concentrations of NDMA was lethal for rats, mice and dogs in other studies.

2.2.1.8 Cancer

No studies were located regarding carcinogenic effects in humans following inhalation exposure to NDMA.

The carcinogenicity of inhaled NDMA has been evaluated in two studies. 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. The 50 ppm concentration is included in Table 2-l and Figure 2-l as an effect level for cancer (cancer effect level, CEL) in rats due to intermittent inhalation exposure of chronic duration.

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. Since the tumors associated with exposure to 0.07 ppm NDMA are consistent with those produced by NDMA in oral and injection studies and the study is reported adequately otherwise, 0.07 ppm is considered to be a CEL for rats and mice due to continuous inhalation exposure of chronic duration (Table 2-1, Figure 2-l).

EPA has adopted the oral carcinogenicity slope factor (BH) of 51 $(mg/kg/day)^{-1}$ (see Section 2.2.2.8) as the slope factor for inhalation (EPA

1988a). The oral slope factor was converted to a unit risk for inhalation of 1.4×10^{-2} $(\bullet g/m^3)^{-1}$, which is equivalent to 42.4 $(ppm)^{-1}$. Using this unit risk, the concentrations associated with upper bound lifetime cancer risk levels of 10^{-4} to 10^{-7} are calculated to be 2.36×10^{-6} to 2.36×10^{-9} ppm, respectively. The cancer risk levels are plotted in Figure 2-l.

2.2.2 Oral Exposure

2.2.2.1 Death

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 two-year period by her husband (Fussgaenger and Ditschuneit 1980, Pedal et al. 1982). It was estimated by the authors that she received at least 4 doses as high as 250-300 mg each, for a total dose of less than 1.5 gram; the mean daily dose was estimated to be 50 •g/kg. Both clinical and autopsy findings indicated that she died of hepatic failure. Two other people (an adult male and a l-year-old boy) died within days after consuming lemonade tainted with unknown quantities of NDMA (Kimbrough 1982, Cooper and Kimbrough 1980). Based on animal studies, the authors estimated that the adult might have received about 1.3 gm, and the boy might have received about 300 mg. In both cases, clinical and autopsy findings primarily showed liver failure and cerebral hemorrhage.

Single dose lethality studies have been conducted in which NDMA was administered to rats and cats by gavage. Druckrey et al. (1967) determined a LD_{50} of 40 mg/kg for rats. This value was determined using an unspecified graphic technique, and confidence limits and specific mortality data were not reported. All of 12 rats that were treated with 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). Jenkins et al. (1985) reported that single 25 mg doses of NDMA resulted in 100% mortality in an unspecified number of rats, but it is unclear if this is dose per kg body weight or dose per total body weight. Single doses of 15 and 20 mg/kg were not lethal for nonpregnant rats but 23 mg/kg was estimated to be the LD_{50} for pregnant rats (Nishie 1983). The LD_{50} for the pregnant rats was extrapolated using mortality of 18-day pregnant rats given single oral doses of 15 or 20 mg NDMA/kg. A dose of 10 mg/kg did not produce deaths in rats within 48 hours (Sumi and Miyakawa 1983). Two of 6 cats died when treated with 50 mg NDMA/kg (Maduagwu and Basir 1980). The NOAEL and appropriate LOAEL values for lethality from these single dose studies are included in the acute duration category in Table 2-2 and Figure 2-2. The 40 mg/kg and 23 mg/kg rat LD_{50}s are also presented in Table 2-2 and Figure 2-2.

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;

TABLE 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine - Oral

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b] (mg/kg/day)	LOAEL[c] (Effect) Less Serious	LOAEL[c] (Effect) Serious	Reference
ACUTE EXPOSURE								
Death								
1	rat	(G)	once				40 (LD_{50})	Druckrey et al. 1967
2, 3	rat	(G)	once		20 (non-pregnant rats)		23 (LD_{50}) (pregnant rats)	Nishie 1983
4	rat	(G)	5-11 days, daily				5 (decreased survival)	Maduagwu and Bassir 1980
5	rat	(G)	once		10			Sumi and Miyakawa 1983
6	rat	(G)	6 days, daily				8 (decreased survival)	McGiven and Ireton 1972
7	mouse	(W)	1 wk daily				9.5 (decreased survival)	Terracini et al. 1966
8	gn pig	(G)	5-11 days, daily				5 (decreased survival)	Maduagwu and Bassir 1980
9	hamster	(W)	1, 2, 4, 7 or 14 d, daily		4.0			Ungar 1984
10	cat	(G)	5-11 days, daily				5 (decreased survival)	Maduagwu and Bassir 1980
11	cat	(G)	once				50 (death)	Maduagwu and Bassir 1980
12	monkey	(G)	5-11 days, daily				5 (decreased survival)	Maduagwu and Bassir 1980

TABLE 2-2 (continued)

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b] (mg/kg/day)	LOAEL[c] (Effect) Less Serious	LOAEL[c] (Effect) Serious	Reference
Systemic								
13	rat	(G)	once	Hepatic		2.5 (degeneration)		Jenkins et al. 1985
14	rat	(G)	once	Hepatic			20 (necrosis)	Nishie 1983
15	rat	(G)	once	Other (thyroid)	20			Nishie 1983
16	rat	(G)	once	Hepatic			8 (necrosis)	Sumi and Miuakawa 1983
17	rat	(F)	1 or 2 wk, daily	Hepatic			3.75 (necrosis)	Khanna and Puri 1966
18, 19	rat	(G)	once	Hepatic	0.7	1.9 (vacuolation)		Korsrud et al. 1973
20	rat	(G)	5-11 d, daily	Hepatic			5 (necrosis)	Maduagwu and Bassir 1980
21	gn pig	(G)	5-11 d, daily	Hepatic			5 (necrosis)	Maduagwu and Bassir 1980
22	hamster	(W)	1, 2, 4, 7 or 14 d, daily	Hepatic		4.0 (portal venopathy)		Ungar 1984
23	cat	(G)	5-11 d, daily	Hepatic			5 (necrosis)	Maduagwu and Bassir 1980
24	monkey	(G)	5-11 d, daily	Hepatic			5 (necrosis)	Maduagwu and Bassir 1980
Immunological								
25	rat	(G)	once		40			Waynforth and Magee 1974

TABLE 2-2 (continued)

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b] (mg/kg/day)	LOAEL[c] (Effect) Less Serious	LOAEL[c] (Effect) Serious	Reference
Developmental								
26	rat	(G)	once, G d 15, or 20				20 (decreased fetal weight)	Nishie 1983
Cancer								
27	mouse	(W)	1 wk, daily				9.5 (CEL[d]) (kidney, lung)	Terracini et al. 1966
INTERMEDIATE EXPOSURE								
Death								
28	rat	(G)	30 d, daily		1			Maduagwu and Bassir 1980
29, 30	rat	(F)	24-110 d daily		2.5		5.0 (death)	Barnes and Magee 1954
31	rat	(F)	40 wk, daily				3.9 (decreased survival)	Magee and Barnes 1956
32	rat	(W)	30 wk, 5 d/wk				0.32 (decreased survival)	Lijinsky and Reuber 1984
33	rat	(G)	30 wk, 2 d/wk				6.0 (decreased survival)	Lijinsky et al. 1987
34	mouse	(W)	49 d, daily				1.8 (decreased survival)	Clapp and Toya 1970
35	mouse	(F)	5 mo, daily				5.26 (decreased survival)	Takayama and Oota 1965
36	mouse	(W)	13 wk, daily				1.9 (decreased survival)	Den Engelse et al. 1974

TABLE 2-2 (continued)

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b] (mg/kg/day)	LOAEL[c] (Effect) Less Serious	LOAEL[c] (Effect) Serious	Reference
37	mouse	(W)	224 d, daily		0.4			Clapp and Toya 1970
38	mouse	(W)	38 wk, daily				1.19 (decreased survival)	Terracini et al. 1966
39	gn pig	(G)	30 d, daily		1			Maduagwu and Bassir 1980
40	hamster	(G)	4 wk, 1 d/wk				10.7 (decreased survival)	Lijinsky et al. 1987
41	hamster	(G)	20 wk, 1 d/wk				5.4 (decreased survival	Lijinsky et al. 1987
42	hamster	(W)	8, 12 or 16 wk, daily				4.0 (death)	Ungar 1986
43	hamster	(W)	28 d, daily		4.0			Ungar 1984
44	cat	(G)	30 d, daily				1 (decreased survival)	Maduagwu and Bassir 1980
45	monkey	(G)	30 d, daily		1			Maduagwu and Bassir 1980
46	mink	(F)	32-34 d, daily				0.32 (decreased survival)	Carter et al. 1969
Systemic								
47	rat	(F)	40 wk, daily	Hepatic			3.9 (necrosis)	Magee and Barnes 1956
48, 49	rat	(F)	62-110 d daily		2.5		5.0 (necrosis)	Barnes and Magee 1954

TABLE 2-2 (continued)

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b] (mg/kg/day)	LOAEL[c] (Effect) Less Serious	LOAEL[c] (Effect) Serious	Reference
50	rat	(F)	4,8 or 12 wk, daily	Hepatic			3.75 (necrosis)	Khanna and Puri 1966
51	rat	(G)	30 d, daily	Hepatic		1 (vacuolation)		Maduagwu and Bassir 1980
52	mouse	(F)	16-92d, daily	Hepatic			13 (hemorrhage/ necrosis)	Otsuka and Kuwahara 1971
53	mouse	(W)	1-4 wk, daily	Hepatic			5.0 (hemorrhage)	Anderson et al. 1986
54	mouse	(F)	5 mo, daily	Hepatic			5.26 (hemorrhage/ necrosis)	Takayama and Oota 1965
55	gn pig	(G)	30 d, daily	Hepatic			1 (necrosis)	Maduagwu and Bassir 1980
56	rabbit	(F)	22 wk, daily	Hepatic		1.6 (fibrosis)		Magee and Barnes 1956
57	hamster	(W)	8, 12 or 16 wk, daily	Hepatic		4.0 (portal venopathy)		Ungar 1986
58	hamster	(W)	28 d, daily	Hepatic		4.0 (portal venopathy)		Ungar 1984
59	dog	(C)	3 wk, 2 d/w (consec)	Hepatic			2.5 (necrosis)	Strombeck et al. 1983
60	cat	(G)	30 d, daily	Hepatic			1 (necrosis)	Maduagwu and Bassir 1980
61	monkey	(G)	30 d, daily	Hepatic			1 (necrosis)	Maduagwu and Bassir 1980
62	mink	(F)	122 d, daily	Hepatic		0.13 (venopathy)		Koppang and Rimeslatten 1976

TABLE 2-2 (continued)

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b] (mg/kg/day)	LOAEL[c] (Effect) Less Serious	LOAEL[c] (Effect) Serious	Reference
63	mink	(F)	32-34 d, daily	Hepatic			0.32 (necrosis)	Carter et al. 1969
Developmental								
64	mouse	(W)	75 d, gestation				0.02 (perinatal death)	Anderson et al. 1978
Cancer								
65	rat	(F)	40 wk, daily				3.9 (CEL[d]) (liver)	Magee and Barnes 1956
66	rat	(W)	30 wk, 5 d/wk				0.3 (CEL[d]) (liver)	Keefer et al. 1973
67	rat	(W)	30 wk, 5 d/wk				0.32(CEL[d]) (liver)	Lijinsky and Reuber 1984
68	rat	(G)	30 wk, 2 d/wk				6.0 (CEL[d]) (liver, lung, kidney)	Lijinsky et al. 1987
69	mouse	(W)	49 d, daily				1.8 (CEL[d]) (liver, lung)	Clapp and Toya 1970
70	mouse	(W)	38 wk, daily				1.19 (CEL[d]) (liver, lung, kidney)	Terracini et al. 1966
71	mouse	(W)	224 d, daily				0.4 (CEL[d]) (liver)	Clapp and Toya 1970
72	mouse	(F)	10 mo, daily				9.04 (CEL[d]) (liver, lung)	Takayama and Oota 1965
73	mouse	(F)	16-92 d, daily				13 (CEL[d]) (lung)	Otsuka and Kuwahara 1971
74	mouse	(G)	50 wk, 2 d/wk				1 (CEL[d]) (liver)	Griciute et al. 1981

TABLE 2-2 (continued)

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b] (mg/kg/day)	LOAEL[c] (Effect) Less Serious	LOAEL[c] (Effect) Serious	Reference
75	mouse	(F)	5 mo, daily				5.26 (CEL[d]) (liver, lung, kidney)	Takayama and Oota 1965
76	hamster	(G)	20 wk, 1 d/wk				5.4 (CEL[d]) (liver)	Lijinsky et al. 1987
77	hamster	(G)	6.5 wk, 2 d/wk				5.4 (CEL[d]) (liver)	Lijinsky et al. 1987
78	hamster	(G)	4 wk, 1 d/wk				10.7 (CEL[d]) (liver)	Lijinsky et al. 1987
79	hamster	(W)	12 or 16 wk, daily				4.0 (CEL[d]) (liver)	Ungar 1986
CHRONIC EXPOSURE								
Death								
80	rat	(F)	54 wk		0.5			Terao et al. 1978
81	mouse	(W)	406 d, daily				0.43 (decreased survival)	Clapp and Toya 1970
82	mink	(F)	321-670 d daily				0.1 (decreased survival)	Koppang and Rimmeslatten 1976
Systemic								
83	rat	(F)	54 wk	Hepatic	0.5			Terao et al. 1978
84	rat	(F)	96 wk, daily	Hepatic	0.5			Arai et al. 1979
85	mink	(F)	321-670 d daily	Hepatic			0.1 (venopathy, focal necrosis)	Koppang and Rimmeslatten 1976

TABLE 2-2 (continued)

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b] (mg/kg/day)	LOAEL[c] (Effect) Less Serious	LOAEL[c] (Effect) Serious	Reference
Cancer								
86	rat	(F)	54 wk daily				0.5 (CEL[d]) (testes)	Terao et al. 1978
87	rat	(F)	96 wk, daily				0.05 (CEL[d]) (liver)	Arai et al. 1979
88	rat	(F)	96 wk, daily				10 (CEL[d]) (liver)	Ito et al. 1982
89	rat	(W)	life, daily				0.02 (CEL[d]) (liver)	Peto et al. 1984
90	mouse	(W)	life, daily				0.43 (CEL[d]) (liver, lung)	Clapp and Toya 1970
91	mink	(F)	321-607 d daily				0.1 (CEL[d]) (liver)	Koppang and Rimeslatten 1976

[a]G - gavage, F - diet, W - drinking water, C - capsule
[b]NOAEL - No Observed Adverse Effect Level
[c]LOAEL - Lowest Observed Adverse Effect Level
[d]CEL - Cancer Effect Level

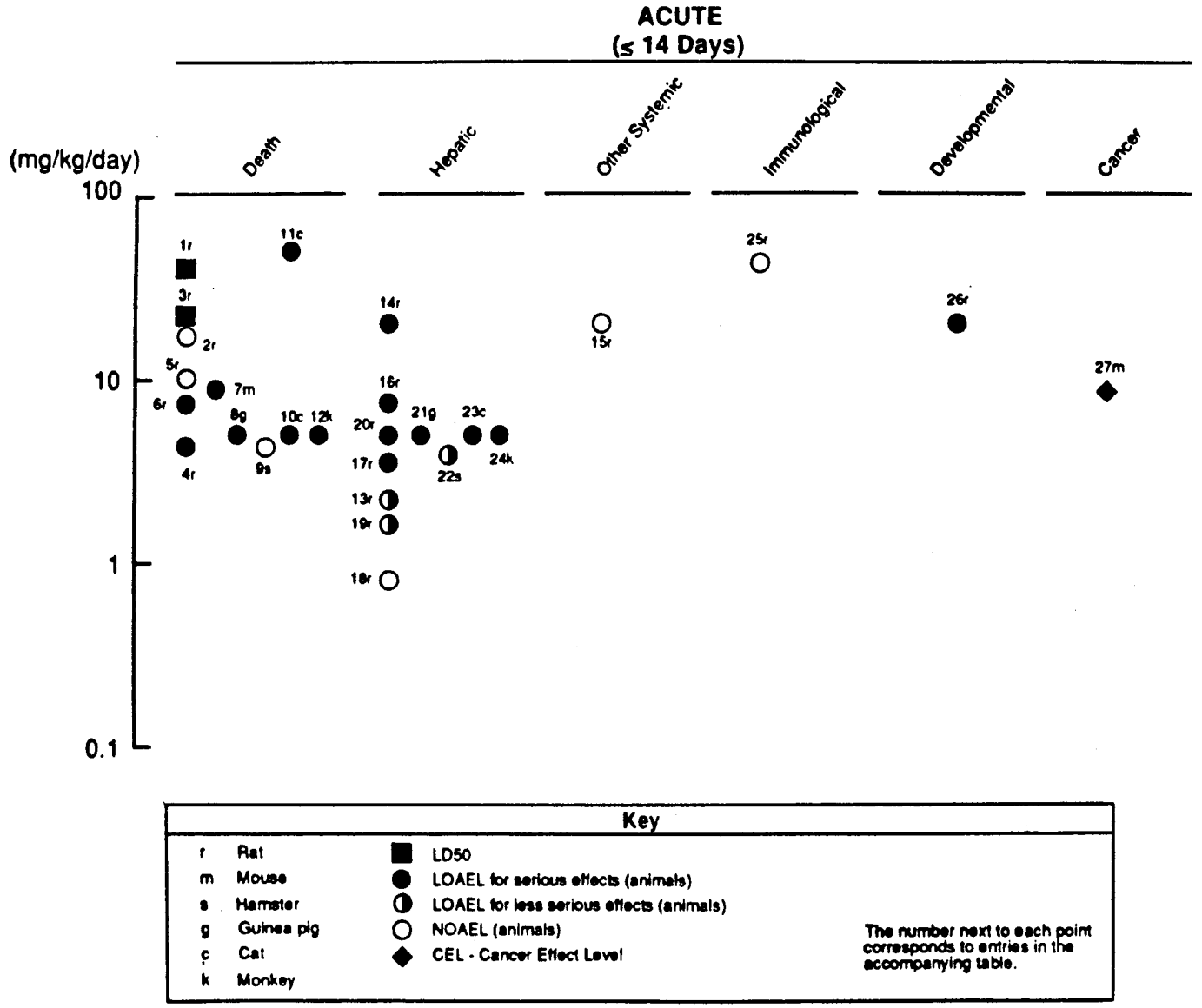

FIGURE 2-2. Levels of Significant Exposure to N-Nitrosodimethylamine - Oral

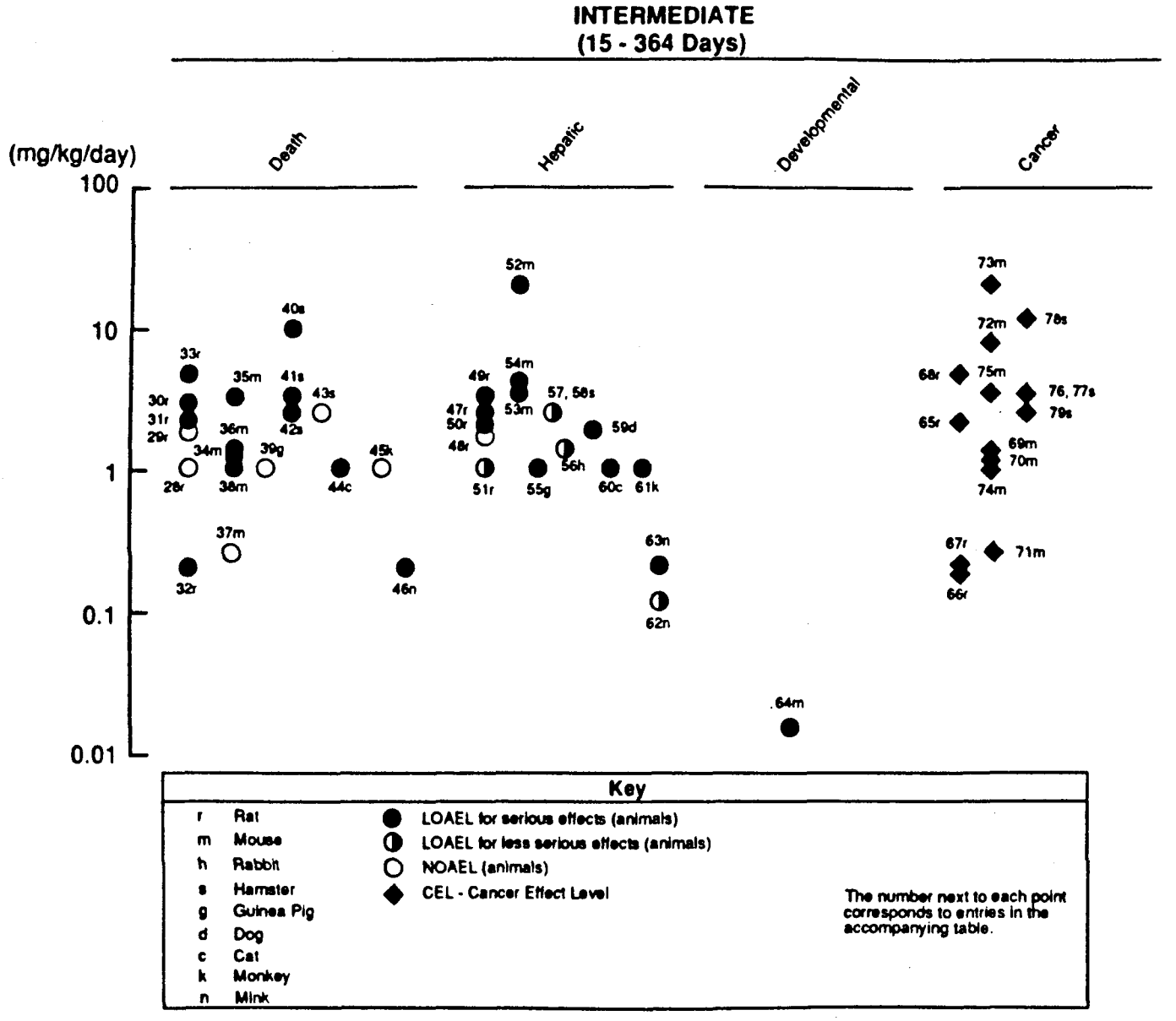

FIGURE 2-2 (continued)

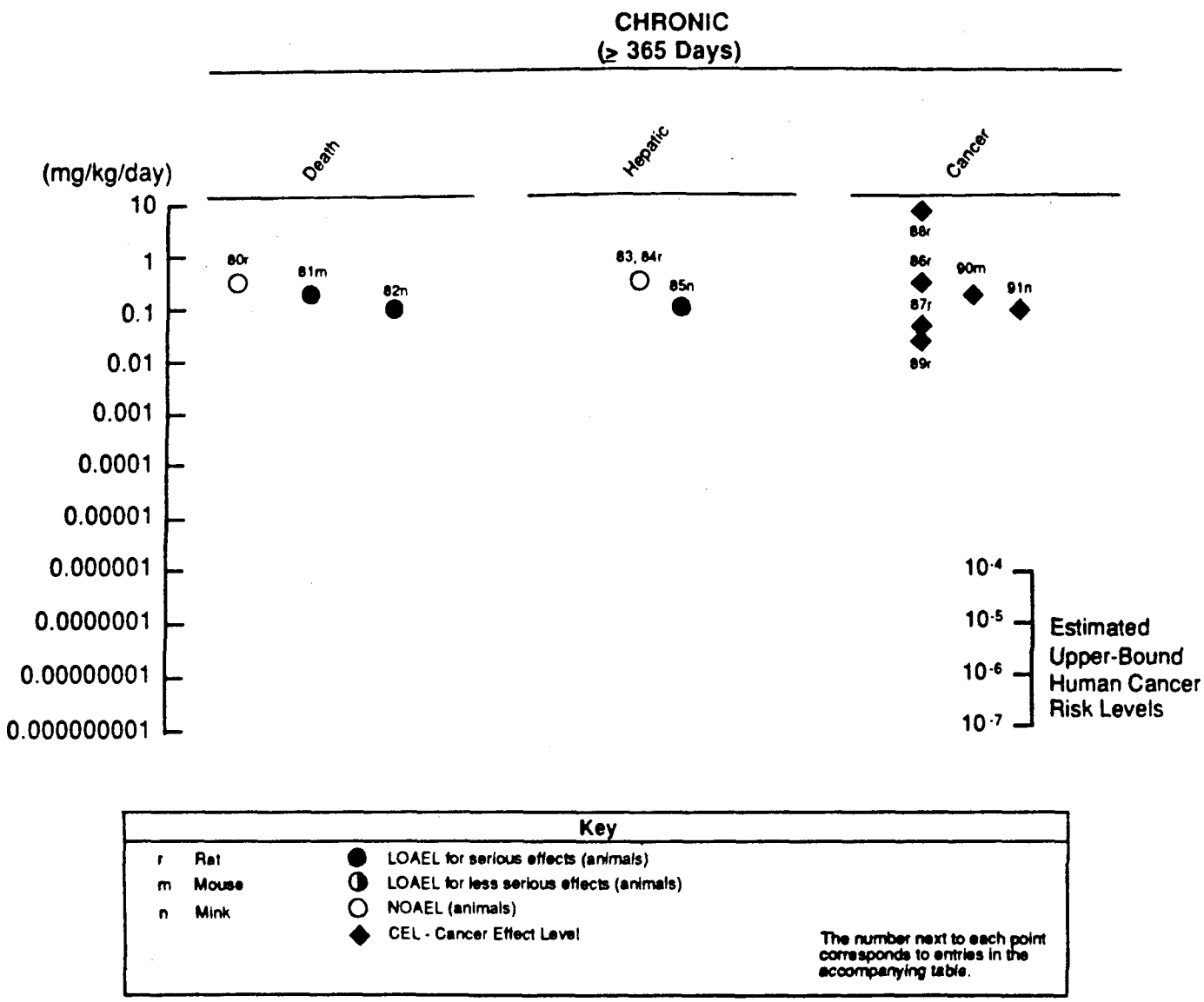

FIGURE 2-2 (continued)

mean survival times were 5-11 days (Maduagwu and Bassir 1980). Rats treated by gavage daily with 8 mg/kg NDMA for 6 days experienced 10% mortality within one month (McGiven and Ireton 1972). Administration of NDMA in the drinking water at a daily dose of 9.5 mg/kg for one week resulted in decreased survival in mice (Terracini et al. 1966). 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). The hamster NOAEL value and all LOAEL values for lethality from these repeated exposure studies are recorded in Table 2-2 and plotted in Figure 2-2. The mouse dose of 9.5 mg/kg/day was calculated from the administered concentration of 50 ppm in water (Terracini et al. 1966); this concentration is presented in Table l-4 for short-term exposure.

Numerous oral studies in which NDMA was administered for intermediate durations (15-365 days) have been conducted. Deaths resulting from intermediate duration exposure to NDMA were usually attributed to liver toxicity or carcinogenicity. Representative lethal and nonlethal intermediate duration exposures in various species are presented below.

In rats, decreased survival resulted when 0.32 mg NDMA/kg was given in the drinking water for 5 days/week for 30 weeks (Lijinsky and Reuber 1984), and when 6 mg/kg was administered by gavage for 2 days/week for 30 weeks (Lijinsky et al. 1987). Control groups were not included in the latter study 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). Daily exposure to 1 mg/kg/day by gavage for 30 days had no effect on survival of rats (Maduagwu and Bassir 1980). 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 dose per total body weight. The NOAEL values and all reliable LOAEL values for lethality in rats from these intermediate duration studies are recorded in Table 2-2 and plotted in Figure 2-2. The rat dose of 5 mg/kg/day was calculated from the administered concentration of 100 ppm in diet (Barnes and Magee 1954); this concentration is presented in Table l-4 for long-term exposure. The rat dose of 0.32 mg/kg/day was calculated from the administered concentration of 5.5 ppm in water (Lijinsky and Reuber 1984); this concentration is also presented in Table l-4 for long-term exposure.

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), 1.9 mg/kg/day via drinking water for 13 weeks (Den Engelse et al. 1974), 1.19 mg/kg/day via drinking water for 38 weeks (Terracini et al. 1966) and 5.26 mg/kg/day via diet for 5 months (Takayama and Oota 1965). Mice that received 0.4 mg/kg/day in drinking water for 224 days did not experience significantly decreased survival (Clapp and Toya

1970). The NOAEL value and all LOAEL values for lethality in mice from these intermediate duration studies are recorded in Table 2-2 and plotted in Figure 2-2.

Survival data for intermediate oral exposure to NDMA are available for species other than rat and mouse. Daily gavage exposure to 1 mg NDMA/kg for 30 days caused decreased survival in cats but not guinea pigs or monkeys (Maduagwu and Bassir 1980). 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); this dose is a NOAEL or LOAEL for lethality depending on duration of exposure. 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 low numbers of animals were tested (three per dose). Mink fed a contaminated diet that provided approximately 0.18 mg NDMA/kg/day died (Martin0 et al. 1988), but there is uncertainty about the dietary concentration of NDMA used to calculate the dose. The mink that were examined in this study were from a commercial breeding colony that died during a 2 month period; durations of exposure were not specified. The NOAEL values and all reliable LOAEL values for lethality in these intermediate duration studies are recorded in Table 2-2 and plotted in Figure 2-2.

Chronic lethality data are available for NDMA-exposed rats, mice and mink. Survival of rats that received 0.5 mg/kg/day of NDMA in the diet for 54 weeks (Terao et al. 1978) was not affected. Decreased survival occurred in mice that were exposed to 0.43 mg/kg/day in the drinking water for life (average 406 days) (Clapp and Toya 1970). Mink appear to be particularly sensitive to NDMA as mortality resulted from ingestion of 0.1 mg/kg/day in the diet for 321-670 days (Koppang and Rimeslatten 1976). The NOAEL value and LOAEL values for lethality in these chronic duration studies are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

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

Respiratory Effects. Petechial and larger hemorrhages were observed in the lungs of two people following lethal poisoning with NDMA (Kimbrough 1982) (Section 2.2.2.1).

Macroscopic congestion was noted in the lungs of rats that were administered 3.75 mg/kg/day doses of NDMA in the diet for l-12 weeks (Khanna and Puri 1966). The adversity of the congestion cannot be determined because results of lung histological examinations were not reported. No studies were

located regarding respiratory effects in animals due to chronic duration oral exposure.

Cardiovascular Effects. Myocardial and endocardial bleeding was observed in a person following lethal poisoning with NDMA (Kimbrough 1982) (Section 2.2.2.1).

Macroscopic congestion was noted in the myocardium of rats that were administered 3.75 mg/kg/day doses of NDMA in the diet for l-12 weeks (Khanna and Puri 1966). The adversity of the congestion cannot be determined because results of heart histological examinations were not reported. No studies were located regarding cardiovascular effects in animals due to chronic duration exposure.

Gastrointestinal Effects. Gastrointestinal hemorrhage occurred in humans following lethal poisoning with NDMA (Kimbrough 1982, Pedal et al. 1982) (Section 2.2.2.1).

NDMA produced similar gastrointestinal effects in animals. 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 (6 in the diet study), and frequency of occurrence was not indicated. Gastrointestinal hemorrhages were also observed in mink that ingested 0.32 or 0.63 mg NDMA/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. No studies were located regarding gastrointestinal effects in animals due to chronic duration exposure.

Hepatic Effects. Five members of a family who consumed unknown quantities of NDMA in lemonade became ill with nausea and vomiting associated with acute liver disease, generalized bleeding and low platelet counts (Kimbrough 1982, Cooper and Kimbrough 1980). As indicated in Section 2.2.2.1, 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) (Section 2.2.2.1). 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.

Hepatotoxicity of NDMA has been described and 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 and hemorrhage often leading to hemorrhagic ascites.

2. HEALTH EFFECTS

In acute studies, characteristic hepatotoxic alterations, as indicated above, occurred in rats following single gavage doses as low as 20 and 8 mg/kg (Nishie 1983, Sumi and Miyakawa 1983), and following daily doses of 3.75 mg/kg in the diet for 1 or 2 weeks (Khanna and Puri 1966). These doses therefore are LOAELs for serious hepatic effects. Jenkins et al. (1985) observed degenerative alterations in the livers of rats following a single 2.5 mg/kg gavage dose of NDMA. As these alterations (collapse of reticulum network in the centrilobular areas followed by regeneration) were nonnecrotic and did not result in loss of the lobular architecture, the 2.5 mg/kg dose is a LOAEL for less serious hepatic effects. Single gavage doses of 1.9 mg/kg and 0.7 mg/kg are a LOAEL for less serious hepatic effects and a NOAEL, respectively, for rats, as 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),. Daily gavage exposure to 5 mg/kg for 5-11 days produced hemorrhagic necrosis in rats, guinea pigs, cats and monkeys (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, a less serious hepatic effect (Ungar 1984). The NOAEL value and LOAEL values for hepatic effects in these acute duration studies are recorded in Table 2-2 and plotted in Figure 2-2. The rat diet dose of 3.75 mg/kg/day was calculated from the administered concentration of 75 ppm in food (Khanna and Puri 1966); this concentration is presented in Table l-4 for short-term exposure. The hamster drinking water dose of 4 mg/kg/day was calculated from the administered concentration of 20 ppm in water (Ungar 1984); this concentration is also presented in Table l-4 for short-term exposure.

In intermediate duration studies with rats, characteristic hepatic effects (described previously) were produced by treatment with NDMA doses of 3.75 mg/kg/day in the diet for 4-12 weeks (Khanna and Puri 1966), 5 mg/kg/day in the diet for 62-95 days (Barnes and Magee 1954), and 3.9 mg/kg/day in the diet for 40 weeks (Magee and Barnes 1956). 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 dose per total body weight. 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), indicating that this dose is a LOAEL for less serious hepatic effects. Hepatic alterations were not observed in rats treated with 2.5 mg/kg/day in the diet for 110 days (Barnes and Magee 1954). The NOAEL and all LOAEL values for hepatic effects in rats from these intermediate duration studies are recorded in Table 2-2 and plotted in Figure 2-2.

Characteristic liver alterations (described previously) occurred in mice that were treated with NDMA doses of 5.0 mg/kg/day in the drinking water for l-4 weeks (Anderson et al. 1986), 13 mg/kg/day in the diet for 16-92 days (Otsuka and Kuwahara 1971) and 5.26 mg/kg/day in the diet for 5 months (Takayama and Oota 1965). These LOAELs for hepatic effects in mice due to intermediate duration exposure are included in Table 2-2 and plotted

in Figure 2-2. The mouse dose of 5.26 mg/kg/day was calculated from the administered concentration of 50 ppm in food (Takayama and Oota 1965); this concentration is presented in Table 1-4 for long-term exposure.

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 (Maduagwa and Basir 1980). Necrotic alterations occurred in dogs treated with 2.5 mg/kg by capsule on 2 days/week for 3 weeks (Strombeck et al. 1983). 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 (Magee and Barnes 1956), indicating that this dose is a less serious LOAEL for hepatic effects. 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), indicating that this dose is also a less serious LOAEL for hepatic effects. 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 (Martin0 et al. 1988); limitations of this study, discussed in Section 2.2.2.1, include uncertainty regarding exposure duration and concentration. All reliable LOAEL values for hepatic effects due to intermediate duration exposure in these studies are recorded in Table 2-2 and plotted in Figure 2-2. The hamster drinking water dose of 4 mg/kg/day was calculated from the administered concentration of 20 ppm in water (Ungar 1984, 1986); this concentration is presented in Table 1-4 for long-term exposure. It should be noted that this water concentration (20 ppm) is higher than the water concentration associated with death (5.5 ppm) due to long-term exposure reported in Table 1-4. The apparent discrepancy in these values is attributable to differences in species sensitivity and length of exposure (rats exposed for 30 weeks, hamsters exposed for 28 days).

In chronic duration studies, characteristic hepatotoxic alterations (described previously) were not observed in rats that were treated with 0.5 mg/kg/day NDMA in the diet for 54 weeks (Terao et al. 1978) or 96 weeks (Arai et al. 1979). Alterations in mink that ingested 0.1 mg/kg/day doses of NDMA in the diet for 321-670 days included occlusive changes in the hepatic veins with focal necrosis (Koppang and Rimeslatten 1976). Data regarding hepatic effects of chronic oral NDMA exposure in other species were not found in the available literature. The NOAEL values and LOAEL value for hepatic effects due to chronic exposure in these studies are recorded in Table 2-2 and plotted in Figure 2-2.

Although hepatotoxicity is the primary effect of NDMA and has been demonstrated in all tested species, calculation of MRLs for NDMA is precluded by insufficient data defining the threshold region (i.e., NOAELs)

for intermediate and chronic exposures, particularly for species which appear to be particularly sensitive (e.g., mink) and because serious effects (perinatal death) occurred in a developmental study (see Section 2.2.2.5) at a dose lower than any NOAELS for liver effects.

Renal Effects. No studies were located regarding renal effects in humans following oral exposure to NDMA.

Limited information is available regarding renal effects of orallyadministered 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, and 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 l-12 weeks (Khanna and Puri 1966). The adversity of the congestion cannot be determined because results of kidney histological examinations were not reported. Moderate tubule congestion with other effects (glomerulus dilatation, slightly thickened Bowman's capsule) were observed in mink that ingested 0.18 mg/kg/day via diet (Martin0 et al. 1988); limitations of this study, discussed in Section 2.2.2.1, include uncertainty regarding exposure duration and concentration.

Other Systemic Effects. Adrenal relative weight and mitotic count were increased in rats following a single 20 mg/kg gavage dose of NDMA (Nishie et al. 1983). Other results of the adrenal histological examinations were not described, precluding assessment of adversity of the increased adrenal weight, There was no effect on thyroid weight or histology in the same study. It therefore is appropriate to regard 20 mg/kg as a NOAEL for thyroid effects in rats due to acute oral exposure (Table 2-2 and Figure 2-2). Macroscopic congestion was noted in spleens of rats that were administered 3.75 mg/kg/day doses of NDMA in the diet for l-12 weeks (Khanna and Puri 1966). The adversity of the congestion cannot be determined because results of spleen histological examinations were not reported.

2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in humans following oral exposure to NDMA.

Limited information is available regarding immunological effects of orally-administered NDMA in animals. Skin graft survival time and white blood cell count were not reduced in rats that received a single 40 mg/kg dose of NDMA by gavage, indicating that treatment was not immunosuppressive (Waynforth and Magee 1974). The dose reported was near the LD50 for rats, but all of the animals died by day 21; it is indicated that the high mortality may partially reflect the stress of skin graft rejection. Although treatment resulted in 100% mortality, this dose represents a NOAEL for immunological effects due to acute duration oral exposure (Table 2-2 and

Figure 2-2). No studies were located regarding immunological effects in animals following intermediate or chronic duration exposure to NDMA.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans following oral exposure to NDMA.

Dogs treated with 2.5 mg NDMA/kg/day by capsule on 2 consecutive days/week for 3 weeks experienced marked central nervous system (CNS) depression (Strombeck et al. 1983). The significance of this observation cannot be ascertained since it was not characterized further. As these dogs developed liver necrosis and hepatic insufficiency, it is possible that the CNS depression is secondary to liver damage rather than a direct neurological effect of NDMA.

2.2.2.5 Developmental Effects

No studies were located regarding developmental effects in humans following oral exposure to NDMA.

Evidence indicates that orally-administered NDMA is a developmental toxicant in animals. Fetuses of rats that received single 20 mg/kg doses of NDMA by gavage on days 15 or 20 of gestation had significantly decreased body weights, but fetal survival data were not reported (Nishie 1983). This dose was also toxic to the dams as indicated by reduced body weight, hepatotoxicity and mortality. Other investigators have reported fetal mortality in rats that were treated with a single 30 mg/kg dose of NDMA by gavage on various days during the first 12 days (Aleksandrov 1974) or 15 days (Napalkov and Alexandrov 1968) of gestation. In other studies, NDMA reportedly caused fetal deaths in rats when administered in the diet at a dose of 5 mg/kg/day from an unspecified day in early pregnancy (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 Alekandrov 1968), or by gavage at a dose of 1.4 mg/kg/day throughout gestation until days 17-21 (not specified) (Napalkov and Alekandrov 1968). Teratogenic effects were not observed in the studies of Aleksandrov (1974) and Napalkov and Alekandrov (1968), and not eva1uated.i.n the studies of Nishie (1983) and Bhattacharyya (1965). Evaluation of the studies of Bhattacharyya (1965), Napalkov and Alekandrov (1968) and Aleksandrov (1974) is complicated by insufficient information regarding experimental design and results; deficiencies include lack of control data, lack of maternal toxicity data, use of pooled data and/or uncertain treatment schedule. Due to these limitations, there is low confidence in the doses associated with fetotoxicity in these studies. As Nishie (1983) is the only adequately reported fetotoxicity study, 20 mg/kg is presented as a LOAEL for developmental effects in rats due to acute exposure to NDMA in Table 2-2 and Figure 2-2.

In another experiment conducted by Aleksandrov (1974), a single dose of 30 mg NDMA/kg was administered by gavage to rats on day 21 of gestation. Histological examination of the offspring at the time of natural death (•274 days after exposure) reportedly showed tumors in 5 of 20 animals. Although this is possibly a manifestation of transplacental carcinogenesis, evaluation of this finding is precluded by limitations including a lack of control data and inadequate reporting of tumor types.

Increased perinatal mortality (stillbirths and newborn deaths) occurred in mice as a consequence of maternal treatment with 0.02 mg NDMA/kg/day in the drinking water (Anderson et al. 1978). The mice were treated for 75 days prior to mating and throughout pregnancy and lactation. Histological examinations of the stillborn fetuses and dead neonates showed no abnormalities. The 0.02 mg/kg/day dose represents a LOAEL for developmental effects due to intermediate duration exposure (Table 2-2 and Figure 2-2).

2.2.2.6 Reproductive Effects

No studies were located regarding reproductive effects in humans following oral exposure to NDMA.

There was no significant increase in time-to-conception in mice that were exposed to 0.02 mg NDMA/kg/day via drinking water for 75 days prior to mating (Anderson et al. 1978). Other reproductive indices were not evaluated.

2.2.2.7 Genotoxic Effects

Methylated DNA (7-methylguanine and O^6 -methylguanine) was detected in the liver of a victim of suspected NDMA poisoning (Herron and Shank 1980). Additional studies regarding genotoxic effects in humans following oral exposure to NDMA were not located.

Oral studies with rats indicate that the liver is sensitive to the genotoxic effects of NDMA. When administered by gavage at a dose of 5.2 mg/kg, NDMA induced damage in rat liver DNA as measured by increased alkaline elution (Brambilla et al. 1981). When administered to rats via diet at a dose of 2.5 mg/kg/day, NDMA induced DNA damage in the liver as measured by a slow sedimentation in alkaline sucrose gradients (Abanobi et al. 1979). The effect was first observed after 2 days of feeding, and became progressively worse during the next 8 weeks of feeding; no proportionate increases in damage occurred when the feedings were continued for 15 or 31 weeks. DNA synthesis and repair was detected in the liver of rats treated with single 10 or 50 mg/kg doses by gavage (Bermudez et al. 1982). Radiolabeled thymidine uptake during mouse testicular DNA synthesis was inhibited by a single gavage dose of 50 mg NDMA/kg (Friedman and Staub 1976).

Administration of NDMA to hamsters by gavage at doses of 50, 100, or 200 mg/kg on the 11th or 12th day of pregnancy caused micronucleus formation and chromosomal aberrations in the embryonic fibroblasts (Inui et al. 1979). NDMA did not induce significant increases in sister chromatid exchanges in bone marrow cells of Chinese hamsters following gavage administration of 12.5-400 mg/kg (Neal and Probst 1983).

2.2.2.8 Cancer

No studies were located regarding carcinogenic effects in humans following oral exposure to NDMA.

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.

Low incidences of epithelial tumors (8.6%) and mesenchymal tumors (14.5%) developed in the kidneys of rats following treatment with 8 mg NDMA/kg/day for 6 days (McGiven and Ireton 1972, Ireton et al. 1972). Evaluation of these data is complicated by the lack of a control group. Daily diet treatment with 9.5 mg/kg for one week produced kidney and lung adenomas in mice (Terracini et al. 1966). No other acute duration oral carcinogenicity studies were found in the reviewed literature. The CEL from the mouse study is presented in the acute duration category in Table 2-2 and in Figure 2-2.

Numerous oral carcinogenicity studies of NDMA of intermediate duration have been conducted. Treatment durations were often in the range of 20-40 weeks, frequency of treatment ranged from once weekly to daily, and carcinogenicity was observed in all studies. Studies representing various treatment durations and various methods of oral treatment (drinking water, diet and gavage) for the lowest doses in different species are identified below.

Rats administered NDMA in the drinking water at doses of 0.3 mg/kg/day, 5 days/week for 30 weeks, developed malignant liver tumors (Keefer et al. 1973, Lijinsky and Reuber 1984). Lijinsky et al. (1987) observed high incidences of liver, lung and kidney tumors in rats that were treated by gavage with 6 mg NDMA/kg twice weekly for 30 weeks; controls were not used in this study. In an intermediate duration diet study with rats, daily treatment with a dose of 3.9 mg/kg for 40 weeks resulted in a 95% incidence of hepatic tumors (Magee and Barnes 1956). The CELs from these

intermediate duration studies with rats are recorded in Table 2-2 and plotted in Figure 2-2.

Liver, lung and/or kidney tumors developed in mice that were exposed to NDMA daily via drinking water at doses of 1.8 mg/kg for 49 days (Clapp and Toya 1970), 1.19 mg/kg for 38 weeks (Terracini et al. 1966) and 0.4 mg/kg for 224 days (Clapp and Toya 1970). Daily administration of NDMA via diet at doses of 13 mg/kg for 16-92 days (Otsuka and Kuwahara 1971), 5.26 mg/kg for 5 months (Takayama and Oota 1965) and 9.04 mg/kg for 10 months (Takayama and Oota 1965) also induced liver, lung and/or kidney tumors in mice. 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). The CELs from these intermediate duration studies with mice are recorded in Table 2-2 and plotted in Figure 2-2.

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, or once weekly with a dose of 5.4 mg/kg for 20 weeks developed high (60-79%) incidences of liver tumors (Lijinsky et al. 1987). However, control groups were not included in the study of Lijinsky et al. (1987). Daily administration of 4 mg/kg in the drinking water to hamsters for 12 or 16 weeks resulted in high incidences of cholangiocellular adenocarcinomas (Ungar 1986). The CELs from these intermediate duration studies with hamsters are recorded in Table 2-2 and plotted in Figure 2-2. 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 (Martin0 et al. 1988); limitations of this study, discussed in Section 2.2.2.1, include uncertainty regarding exposure duration and concentration, examination only of animals that died and use of historical controls. Due to the limitations of this study, it is inappropriate to present a CEL for mink due to intermediate duration exposure in Table 2-2 and Figure 2-2.

Chronic oral carcinogenicity studies of NDMA have been conducted with rats, mice and mink. Tumors at sites other than the liver and testis have not been associated with chronic treatment. *Terao et* al. (1978) observed a 47% increase in the incidence of testicular Leydig-cell tumors, but no tumors in the liver or other tissues, in rats that were treated with 0.5 mg/kg daily doses of NDMA in the diet for 54 weeks. Increased incidences of liver tumors, but not testicular interstitial cell tumors, occurred in rats that received 0.05 or 0.5 mg/kg/day doses of NDMA in the diet for 96 weeks (Arai et al. 1979). In this study, liver tumor incidences were generally higher in female rats than in male rats. Increased incidences of liver tumors also occurred in rats that were treated with NDMA in the diet for 96 weeks at a dose of 10.0 mg/kg/day (Ito et al. 1982); similar treatment with doses of 0.1 or 1.0 mg/kg/day did not produce increased incidences of liver tumors. It should be noted that Wistar rats were tested in both the Ito et al. (1982) and Arai et al. (1979) studies. The reason for the lack of liver tumors at doses below the relatively high 10 mg/kg/day dose in the Ito et al. (1979) study is not clear, but may be related to low susceptibility of

male rats. In a lifetime drinking water study, Peto et al. (1984) administered doses of 0.001-1.2 mg/kg/day to rats and observed that incidences of liver tumors were significantly increased at ≥0.02 mg/kg/day; median survival time at the lowest tumorigenic doses was in the range of 28-31 months. Crampton (1980) administered NDMA to rats in the drinking water at doses ranging from 0.002-1.5 mg/kg/day for life and observed increased liver tumor incidences at ≥0.008 mg/kg/day; median survival time at 0.008 mg/kg day was >900 days. The results reported by Crampton (1980) were preliminary and there is uncertainty regarding the dosages; ppm concentrations in water and mg/kg/day equivalency were reported, but the basis for the equivalency is not indicated and the conversion cannot be verified using standard methodology. Clapp and Toya (1970) administered NDMA to 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). The CELs for rats, mice and mink from these chronic studies, except the uncertain value from the Crampton (1980) study, are recorded in Table 2-2 and plotted in Figure 2-2.

The EPA (1988a) has derived and verified an oral slope factor (B_H) of 51 $(mg/kg/day)^{-1}$ for NDMA based on the liver tumor response in the Peto et al. (1984) study. Using this slope factor, the doses associated with upper bound lifetime cancer risk levels of 10^{-4} to 10^{-7} are calculated to be 1.96×10^{-6} to 1.96×10^{-9} mg/kg/day, respectively. The cancer risk levels are plotted in Figure 2-2.

2.2.3 Dennal Exposure

2.2.3.1 Death

No studies were located regarding lethality in humans or animals following dermal exposure to NDMA.

2.2.3.2 Systemic Effects

No studies were located regarding systemic effects in humans following dermal exposure to NDMA. Limited information was located regarding systemic effects in animals following dermal exposure; no animal studies provided information on respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic or renal effects.

Dermal/Ocular Effects. Small ulcerations and scarring of the skin were observed in hairless mice that were treated once weekly with topical doses of 33.3 mg NDMA/kg for 20 weeks (Iversen 1980). No studies were located regarding NDMA-related ocular effects in animals.

Other Systemic Effects. Barnes and Magee (1954) noted that daily application of 100 mg NDMA/kg to rats for 4 days had no effect on general condition.

No studies were located regarding the following effects in humans or animals following dermal exposure to NDMA:

2.2.3.3 Immunological Effects

2.2.3.4 Neurological Effects

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

2.2.3.8 Cancer

No studies were located regarding carcinogenic effects in humans following dermal exposure to NDMA.
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 8 to a cage and could have licked the NDMA off each other or inhaled the compound due to its volatility.

2.3 RELEVANCE TO PUBLIC HEALTH

Death. Oral LD_{50} values of 23 and 40 mg NDMA/kg have been reported for pregnant and nonpregnant rats, respectively (Nishie 1983, Druckrey 1967). Oral LD_{50}s have not been determined for NDMA in other species. LD_{50}s for single doses of NDMA administered by intraperitoneal injection have also been reported; these values are consistent with the oral LD_{50}s and include 26.5 mg/kg in rats (Barnes and Magee 1954), 42.7 mg/kg in rats (Heath 1962) and 19 mg/kg in mice (Friedman and Sanders 1976). Repeated oral exposure to NDMA resulted in decreased survival in rats, mice and all other species that have been tested. In general, doses ranging from approximately 0.1-5 mg/kg/day have produced death in animals after several days to several months of exposure. Variations in lethal doses appear to be attributable more to intraspecies differences than differences in frequency or method of oral treatment. With the exception of mink, there do not appear to be marked differences in sensitivity among the species that have been tested. Deaths resulting from a single exposure or repeated exposures for several days or several weeks are generally attributed to liver toxicity; deaths associated

with longer duration (e.g., 20-40 weeks) exposures are due to liver tumor development.

In general, lethal doses,of NDMA and causes of death are similar among animal species. Human fatalities due to ingestion or inhalation of NDMA were also attributed to liver toxicity but adequate dose information is not available.

Systemic Effects. Hepatotoxicity is the primary systemic effect of NDMA. Hepatotoxicity has been demonstrated in all animal species that have been tested, and has been observed in humans who were exposed to NDMA by ingestion or inhalation. The characteristic hemorrhagic necrosis caused by NDMA are particularly prevalent following exposure to acutely toxic single doses or repeated doses for short durations. Liver tumors are the predominant effect of longer duration exposures. The mechanism of NDMAinduced liver toxicity is not clearly understood but may be related to alkylation of cellular protein (Barnes and Magee 1954, Magee et al. 1976, Diaz Gomez et al. 1981, 1983, Martin0 et al. 1988).

Although the hepatotoxicity of NDMA has been established unequivocally in numerous acute, intermediate and chronic duration oral studies with animals, relatively few of the studies delineate dose-response relationships and appropriate information regarding thresholds for this effect is not available. As noted for lethality, reported hepatotoxic doses for all species occur in the same general range with variations attributable more to intraspecies differences than treatment schedule or method. Human fatalities due to oral and inhalation exposure to NDMA have been reported in which hemorrhagic, necrotic and cirrhotic alterations in the liver were observed, indicating that NDMA produces similar hepatic effects in humans and animals. Therefore it is reasonable to expect that NDMA also will be hepatotoxic in humans at sublethal doses.

Limited information is available regarding nonhepatic systemic effects of NDMA in humans. This information has been obtained from autopsies of victims accidentally exposed to NDYA vapors or poisoned after ingestion of NDMA. The effects can be described as a general bleeding tendency. Hemorrhages have been noticed in the gastrointestinal tract, heart, respiratory system and brain (Freund 1937; Kimbrough 1982). The mechanism by which NDMA could induce bleeding is not known, but the bleeding tendency could be a consequence of decreased formation of clotting factors resulting from liver damage, impairment of the clotting mechanism or decreased number or function of platelets. Jacobson et al. (1955), for example, showed that NDMA greatly increases prothrombin time in dogs exposed to NDMA by inhalation. It is also possible that hemorrhagic effects could be caused by effects of NDMA on tissues. Because of its irritant properties, it is not difficult to explain the occurrence of hemorrhages in tissues that have direct contact with NDMA (gastrointestinal bleeding after oral ingestion, or bleeding of the bronchi and trachea after inhalation). However, it remains unknown why gastrointestinal bleeding can occur following inhalation

exposure. An alternative explanation could be that NDMA has a direct effect on the blood vessels. In fact, Ungar (1984, 1986) showed that oral treatment of hamsters with NDMA induced fragmentation of elastic fibers in portal vessels as well as denudation of the portal endothelium. Furthermore, autopsy of a victim of acute NDMA poisoning showed that "the central hepatic veins had lost their endothelial'lining cells" (Kimbrough 1982).

There is a relative paucity of information for nonhepatic systemic effects in animals because the emphasis of most studies was on hepatotoxicity or cancer, for which the liver is the primary target organ. Nonhepatic systemic effects that have been reported include gastrointestinal hemorrhage and congestion of several organs (kidney, lung, heart, spleen) in rats and/or mink, but the prevalence of these effects cannot be determined because these sites were examined infrequently.

Immunological Effects. A single oral dose of NDMA near the oral LD_{50} did not reduce humoral immune response in rats, but a single intraperitoneal dose near the intraperitoneal LD_{50} reduced humoral immune response in mice (Waynforth and Magee 1974). A number of other recent studies have found that NDMA given by intraperitoneal injection alters humoral immunity and antibody-mediated host defense mechanisms (Kaminski et al. 1989; Thomas et al. 1985, Myers et al. 1986, 1987, Scherf and Schmahl 1975, Holsapple et al. 1983, 1984, 1985, Johnson et al. 1987a,b). Immunosuppression resulting from NDMA exposure is not believed to be a result of direct interaction between the reactive intermediaries of NDMA and splenic lymphocytes, thereby indicating a difference between the mechanisms of immunotoxicity and carcinogenicity/genotoxicity (Holsapple et al. 1984). In vivo studies have shown that NDMA modulates the cellular immune response by altering the production and/or maturation/differentiation of bone marrow stem cells into functional macrophages (Myers et al. 1986, 1987). In vitro tests identify the primary cell target of NDMA as the B-lymphocyte (Holsapple et al. 1984, 1985). Thus, it is likely that NDMA decreases the overall reactivity of both T- and B-lymphocytes. It is not known whether NDMA is likely to be immunosuppressive in humans.

Developmental Effects. NDMA was fetotoxic to rats at oral doses that were toxic to the mother. Limited data indicate that these doses were not teratogenic for the rats. Oral administration of NDMA to mice resulted in increased perinatal deaths without histological abnormalities. It is not known whether NDMA could cause developmental effects in humans, but it cou be a potential developmental toxicant at doses which are toxic to pregnant women.

Reproductive Effects. Mice that were exposed to NDMA in drinking water prior to mating and during pregnancy and lactation showed an increase in the frequency of perinatal death among their offspring. Based on these data, NDMA could be considered a potential human reproductive toxicant.

2. HEALTH EFFECTS

Genotoxic Effects. Several in vitro studies have examined genotoxic effects of NDMA in human cells. As indicated in Table 2-3, NDMA induced DNA repair and synthesis in human lymphoblasts, and sister chromatid exchange in human lymphocytes and fibroblasts.

Genotoxicity of NDMA has been demonstrated consistently in numerous in vitro studies with non-human systems. As indicated in Table 2-3, NDMA was mutagenic in bacteria (Salmonella tvnhimurium, Escherichia coli), yeast (Saccharomvces cerevisiae), and mammalian cells (Chinese hamster V79 and ovary cells and mouse lymphoma L5178Y cells). NDMA induced unscheduled DNA synthesis and DNA repair and synthesis in rat, mouse and hamster hepatocytes. Treatment-related DNA fragmentation occurred in rat and human hepatocytes. Chromosomal aberrations occurred in Chinese hamster primary lung cells, rat ascites hepatoma cells, and rat esophageal tumor cells. Sister chromatid exchanges occurred in Chinese hamster ovary cells, Chinese hamster primary lung cells, human lymphoblasts and fibroblasts, and rat esophageal tumor and ascites hepatoma cells.

In vivo studies (Table 2-4) have shown that NDMA methylates DNA, causes DNA fragmentation and induces DNA synthesis and repair in liver and other tissues of various species (e.g., rat, mouse, hamster, gerbil). NDMA induced chromosomal aberrations in hamster embryonic fibrolasts, sister chromatid exchanges in mouse bone marrow cells, and micronuclei in rat hepatocytes and rat and mouse bone marrow cells. The genotoxic effects indicated above occurred after inhalation, oral or intraperitoneal administration of NDMA. Sperm abnormalities were not seen in mice following intraperitoneal administration of NDMA. Sex linked recessive lethal mutations occurred in Drosophila melanogaster, which indicates potential heritable mutagenicity of NDMA.

The weight of evidence indicates that NDMA is genotoxic in mammalian cells. In vitro studies with human cells, as well as in vitro and in vivo studies with animals and microbes, support this conclusion. Given the type and weight of genotoxicity evidence, it is appropriate to predict that NDMA poses a genotoxic threat to humans.

Cancer. The oral carcinogenicity of NDMA has been demonstrated in numerous studies with various species of animals. Inhalation exposure to NDMA has been reported to be carcinogenic to rats and mice in two studies. The carcinogenicity of NDMA is also documented in numerous single and or weekly subcutaneous and intraperitoneal injection studies, and in studies in which NDMA was administered prenatally and to newborn animals. Many of the carcinogenicity studies of NDMA were conducted specifically to induce cancer for various purposes, such as investigations of structure-activity relationships and pathogenesis. Tumors in tissues other than the liver and respiratory system (e.g., kidney, testis) have not been observed often in many of the carcinogenicity studies; this appears to be attributable in part to limited examination of nonhepatic tissues.

TABLE 2-3. Genotoxicity of N-Nitrosodimethylamine In Vitro

Endpoint	Species (Test System)	Result: With Activation	Result: Without Activation	References
Gene mutation	Salmonella typhimurium	+	+	Araki et al. 1984, *Bartsch et al. 1980, Langenbach et al. 1986,* DeFlora et al. 1984, Prival and Mitchell 1981, Ishidate and Yoshikawa 1980
	Escherichia coli	+	NT	Araki et al. 1984, DeFlora et al. 1984
	Saccharomyces cerevisae	+	NT	Jagannath et al. 1981, Frezza et al. 1983
	Chinese Hamster V79 and ovary cells	+	-	Kuroki et al. 1977, Adair and Carver 1983, O'Neill et al. 1982, Carver et al. 1981, Dickins et al. 1985, Bartsch et al. 1980, Katoh et al. 1982, Langenbach 1986, Hsie et al. 1978
	Mouse lymphoma L5178Y cells	+	-	Amacher and Paillet 1983, Clive et al. 1979
DNA fragmentation	Rat hepatocytes	NT	+	Bradley et al. 1982
	Human hepatocytes	NT	+	Martelli et al. 1985
Chromosomal aberrations	Chinese hamster lung cells	+	NT	Matsuoka et al. 1979, 1986, Ishidate and Yoshikawa 1980
	Rat ascites hepatoma (AH66B) and rat esophageal (R1, R3) tumor cells	NT	+	Ikeuchi and Sasaki 1981

TABLE 2-3 (continued)

Endpoint	Species (Test System)	Result: With Activation	Result: Without Activation	References
Sister-chromatid exchange	Rat esophageal tumor, ascites hepatoma	NT	+	Abe and Sasaki 1982, Ikeuchi and Sasaki 1981
	Human lymphocytes	+	-	Inoue et al. 1983, Madle et al. 1987,
	Human fibroblasts	+	NT	Tomkins et al. 1982
	Chinese hamster ovary cells	+	NT/-	Tomkins et al. 1982, Okinaka et al. 1981 Blazak et al. 1985
	Chinese hamster V79 cells	+	-	Madle et al. 1987, Sirianni and Huang 1987 Blazak et al. 1985
	Chinese hamster primary lung cells	+	-	Shimizu et al. 1984
DNA Damage	Rat hepatocytes	NT	+	Bermudez et al. 1982
DNA repair/ synthesis	Rat hepatocytes	+	+	Andrae and Schwarz 1981,
	Human lymphoblasts	+	NT	Andrae et al. 1979
	Mice hepatocytes	NT	+	McQueen et al. 1983
	Hamster hepatocytes	NT	+	McQueen et al. 1983
	Rat pancreatic cells	NT	-	Steinmetz and Mirsalis 1984

NT = Not tested

TABLE 2-4. Genotoxicity of N-Nitrosodimethylamine In Vivo

Endpoint	Species (Test System)	Result	References
DNA methylation	Rat, mouse, hamster and/or gerbil liver	+	O'Connor et al. 1982, Bamborschke et al. 1983, Pegg et al. 1981, Pegg and Hui 1978, Stumpf et al. 1979
	Human liver	+	Herron and Shank 1980
DNA fragmentation	Rat liver and kidney elution	+	Brambilla et al. 1981, Petzold and Swenberg 1978, Abanobi et al. 1979, Bermudez et al. 1982
	Mouse liver and kidney elution	+	Cesarone et al. 1982
DNA synthesis and repair	Fetal mouse kidney and liver	+	Bolognesi et al. 1988
	Mouse testes	+	Friedman and Staub 1976, Cesarone et al. 1979
	Rat liver	+	Bakke and Mirsalis 1984, Kornbrust and Dietz 1985, Doolittle et al. 1984
	Rat respiratory cells	+	Doolittle et al. 1984
	Rat spermatocytes	-	Doolittle et al. 1984
Sex-linked recessive lethal mutations	Drosophila melanogaster	+	Brodberg et al. 1987, Blount et al. 1985, Lee et al. 1983
Sperm abnormalities	Mouse	-	Wyrobek and Bruce 1975
Sister chromatid exchange	Chinese hamster bone marrow	+/-	Neal and Probst 1983
	Mouse bone marrow	+	Sharma et al. 1983, Bauknecht et al. 1977
Chromosome aberrations	Hamster embryonic fibroblasts	+	Inui et al. 1979
Micronucleus	Rat bone marrow	+/-	Trzos et al. 1978
	Rat hepatocytes	+	Mehta et al. 1987, Tates et al. 1980
	Mouse bone marrow	+	Odagiri et al. 1986, Bauknecht et al. 1977
	Hamster embryonic fibroblasts	+	Inui et al. 1983

2. HEALTH EFFECTS

There is increasing evidence, derived from in vitro and in vivo metabolic studies, indicating that the carcinogenic effects of NDMA are due to a metabolite rather than the compound itself (Singer 1979). NDMA is converted into an alkylating (methylating) agent after metabolism by microsomal mixed-function oxidases. This process occurs principally in the liver and to a lesser extent in kidney and lungs, and results in the methylation of cellular macromolecules such as DNA, RNA and other proteins. Methylation occurs at several positions in DNA including N^{-1}, N^{-3} or N^{-7} of deoxyadenosine; N^{-3}, N^{-7} or 0^{6} of deoxyguanosine; N^{-3} of deoxycytidine; and 0^{2} or 0^{4} of thymidine. Experimental evidence indicates that methylation at the 0^{6}-position of guanine may be responsible for the carcinogenic activity of nitrosamines in general, however, the carcinogenic potential of other methylated products cannot be ruled out. The methylation of DNA by NDMA has been studied extensively (e.g., Bamborschke et al. 1983, O'Connor et al. 1982, Pegg et al. 1981, Pegg and Hui 1978, Stumpf et al. 1979.).

The carcinogenic properties of NDMA, and nitrosamines in general, have been extensively studied. It is of considerable interest that, despite its ubiquitous distribution, NDMA induces tumors in a limited number of organs and tissues and that there are marked differences in this response among animal species. Differences in pharmacokinetics properties seem to play an important role in the carcinogenic action of NDMA (Pegg 1980, Lijinsky 1987). For example, the degree of hepatic extraction from the portal blood seems to determine whether tumors develop in extrahepatic sites. Therefore, large doses of NDMA tend to induce extrahepatic tumors (spill-over effect). In addition, metabolic activating systems and repair mechanisms may not operate at the same rates in different organs and different species. Route of administration also seems to be a factor in NDMA carcinogenesis since different responses are seen in a particular species when different routes of exposure are used. This suggests that rates of absorption can determine the site of tumor development.

Based on the unequivocal evidence of carcinogenicity in animals, it is reasonable to anticipate that NDMA will be carcinogenic in humans. It is important to recognize that this evidence also indicates that oral exposures of acute and intermediate duration are sufficient to induce cancer.

2.4 LEVELS IN HUMAN TISSUES AND FLUIDS ASSOCIATED WITH HEALTH EFFECTS

A considerable amount of DNA methylation in the liver of a suspected NDMA poisoning case was reported by Herron and Shank (1980). Based on studies in rats, in which the amount of DNA alkylation could be correlated with known amount of orally administered NDMA, the authors estimated that the victim had been exposed to a dose of 20 mg/kg or more of NDMA. No other studies were located regarding levels of NDMA or its metabolites in human tissues and fluids associated with effects. Several analytical methods have been developed to determine levels of NDMA in human tissues and fluids. These methods are described in Chapter 6.

2. HEALTH EFFECTS

2.5 LEVELS IN THE ENVIRONMENT ASSOCIATED WITH LEVELS IN HUMAN TISSUES AND/OR HEALTH EFFECTS

Although data relating specific amounts of NDMA in the environment with levels in human tissues and/or health effects have not been reported, some qualitative information is available. This information, given below, has to be interpreted with caution since results were not always rigorously reported or evaluated, and endogenous formation of NDMA was not quantitated.

A high incidence of nasopharyngeal carcinoma was found in Tunisia (North Africa), Southern China and Greenland among populations who consume foods with a high content of volatile nitrosamines (Poirier et al. 1987). Lu et al. (1987) found a positive correlation between the amount of NDMA and other nitrosamines in the gastric juice of Chinese with a high incidence of esophageal carcinoma. Wild et al. (1987) observed a positive relationship between levels of 0^6-methyldeoxyguanosine (implicated in the initiating process of nitrosamine-induced cancer) and incidences of esophageal and stomach cancer in the province of Lin-xian, China. Yu and Henderson (1987) reported finding a high incidence of nasopharyngeal carcinoma in individuals from Hong Kong, who are known to consume from early in life considerable amounts of Cantonese-style salted fish, which has a high content of NDMA and other nitrosamines.

2.6 TOXICOKINETICS

2.6.1 Absorption

2.6.1.1 Inhalation Exposure

No studies were located regarding the rate and extent of absorption of NDMA following inhalation exposure of humans or animals to NDMA. However, it can be inferred that NDMA is absorbed from the air since it can be detected in the urine of rats (Klein and Schmezer 1984) and dogs (Raabe 1986) after inhalation exposure. Absorption is also indicated by reports of human deaths following inhalation of NDMA (see Section 2.2.1.1).

2.6.1.2 Oral Exposure

No studies were located regarding the absorption of NDMA following oral exposure of humans.

The absorption of NDMA from the gastrointestinal tract of animals is fast. Less than 2% of the labelled compound could be recovered from the gastrointestinal tract 15 minutes after oral administration of 14C-NDMA to rats (Diaz Gomez et al. 1977). Absorption seems to be independent of the dose administered (Diaz Gomez et al. 1972). In the rat, NDMA is absorbed much faster from the small intestine than from the stomach, in isolated preparations (Heading et al. 1974) and in vivo (Pegg and Perry 1981).

Ishiwata et al. (1977) reported that the disappearance curve of NDMA from isolated guinea pig stomach and small intestine follows first order kinetics.

2.6.1.3 Dermal Exposure

No studies were located regarding the absorption of NDMA following dermal exposure of humans.

Indirect evidence indicating that NDMA may be absorbed through the skin was found in a study published by Iversen (1980) in which topical application of NDMA induced lung adenomas in mice. The results from Iversen, however, should be interpreted with caution since the mice were housed 8 to a cage and could have licked the NDMA from each other and also could have inhaled this volatile compound.

2.6.2 Distribution

Unmetabolized NDMA was found to be evenly distributed among the main organs of mice and rats shortly after i.v. injection to animals in which the metabolism of NDMA had been inhibited (Magee 1956; Johansson and Tjalve 1978). Wishnok et al. (1978) reported a similar finding in rats following i.p. injections. Johnson et al. (1987a) reported that one hour after a dose of 6 mg ^{14}C-NDMA/kg was administered by intraperitoneal injection to mice, the liver contained two times as much radioactivity as the kidney, spleen and thymus.

2.6.2.1 Inhalation Exposure

No studies were located regarding the distribution of NDMA following inhalation exposure of humans or animals.

2.6.2.2 Oral Exposure

No studies were located regarding the distribution of NDMA following oral exposure of humans.

Daugherty and Clapp (1976) reported that 15 minutes after oral administration of ^{14}C-NDMA to mice, the relative amounts of radioactivity in the homogenates of heart, forestomach, esophagus, liver and lung were 1, 2, 3, 10 and 70, respectively. The differences could be attributed to different tissue affinity, transport and/or metabolism. Measurable amounts of NDMA were reported in blood, liver, kidney, lungs and brain of mice exposed to 5 mg NDMA/kg/day in drinking water.for up to 4 weeks (Anderson et al. 1986). NDMA has been detected in maternal blood, placenta, fetus and amniotic fluid of pregnant Syrian hamsters for up to 2 hours after a single subcutaneous dose of 12.5 mg/kg of the chemical (Althoff et al. 1977).

Liver and kidney DNA from 14-day-old rats became labelled after treating the nursing mothers with ^{14}C-NDMA by gavage (Diaz Gomez et al. 1986).

2.6.2.3 Dermal Exposure

No studies were located regarding the distribution of NDMA following dermal exposure of humans.

The study by Iversen (1980), in which lung adenomas were noticed in mice after skin application of NDMA, indicates that this chemical (or a metabolite) was distributed to the lungs.

2.6.3 Metabolism

Evidence from in vitro and in vivo studies with rodents indicates that NDMA is metabolized by hydroxylation of the a-carbon, followed by formation of formaldehyde, molecular nitrogen and a methylating agent, which is considered to be the carcinogenic form (Lotlikar et al. 1975; Czygan et al. 1973). Recent evidence suggests that a significant proportion of NDMA is metabolized via a denitrosation mechanism. The latter mechanism takes place in rats in vivo, as indicated by the urinary excretion of labelled methylamine after i.v. administration of ^{14}C-NDMA (Keefer *et* al. 1987), and in human liver microsomes (Yoo et al. 1988). The metabolism of NDMA is summarized in Figure 2-3.

Metabolism of NDMA varies among species (Prassana et al. 1985; Montesano et al. 1982). Age of the animal and route of administration can also influence the rate of metabolism of NDMA (Phillips et al. 1975). In addition, at varying doses, different forms of enzymes appear to be responsible for NDMA metabolism (Kroeger-Koepke and Michejda 1979; Lotlikar et al. 1978).

2.6.3.1 Inhalation Exposure

No studies were located regarding the metabolism of NDMA following inhalation exposure of humans or animals.

2.6.3.2 Oral Exposure

No studies were located regarding the metabolism of NDMA following oral exposure of humans.

Phillips et al. (1975) demonstrated that NDMA is metabolized at a lower rate when given orally to rats than when administered by parenteral routes.

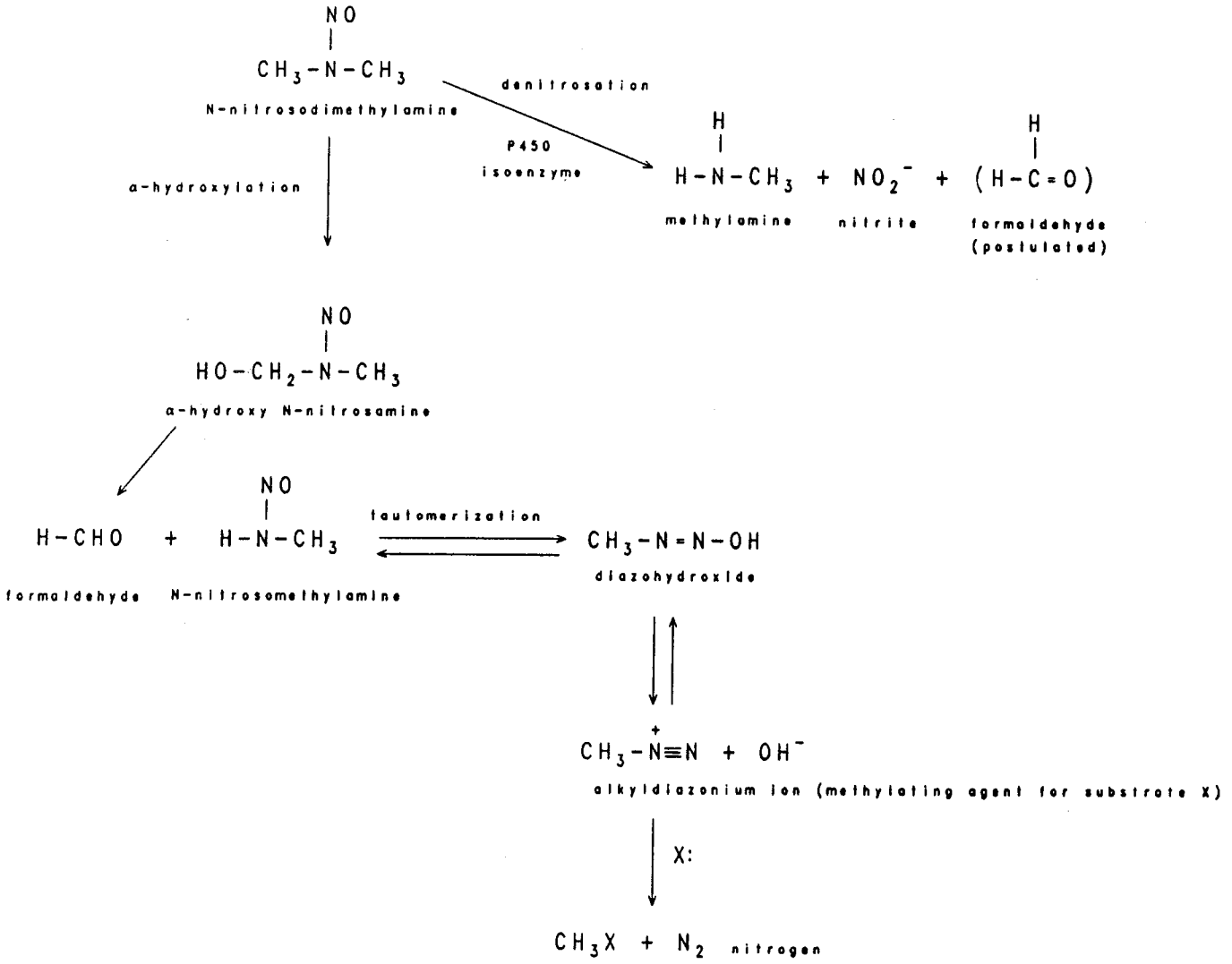

Figure 2-3. Metabolism of N-Nitrosodimethylamine

Source: Crygan et al. 1973; Keefer et al., 1987; Lotikar et al. 1975; Yoo et al. 1988

2.6.3.3 Dermal Exposure

No studies were located regarding the metabolism of NDMA following dermal exposure of humans or animals.

2.6.4 Excretion

Labelled $C0_2$ can be detected in the exhaled air 1 hour after i.p. administration of ^{14}C-NDMA to rats (Phillips et al. 1975). Hemminki (1982) administered labelled NDMA by intraperitoneal injection to rats and was able to detect three main radioactive fractions in the urine over a period of 5 days. Fraction I was composed of radioactive aminoacids, fraction II of allantoin and a metabolite of thiazolidine-4-carboxylic acid, and fraction III of 7-methylguanine.

2.6.4.1 Inhalation Exposure

Klein and Schmezer (1984) reported that 10-30% of NDMA is excreted by exhalation after exposing rats to the chemical during 10 minutes by endotracheal intubation. In beagle dogs, 23% of the administered radioactive label is exhaled in 30 minutes after a 3 hour inhalation exposure (Raabe 1986).

2.6.4.2 Oral Exposure

Spiegelhalder et al. (1982) reported that, in a 24 hour period, human volunteers excreted in the urine between 0.5 and 2.4% of an ingested dose of 12-30 pg of NDMA added to drinking fluids containing ethanol.

Unchanged NDMA was recovered in the urine and feces of rats up to 24 hours after a single oral dose of 50 mg (Magee 1956). Swann et al. (1984) did not detect labelled NDMA in the urine of rats after oral administration of 30 μg/Kg of ^{14}C-NDMA in water. Phillips et al. (1975 determined that after administration of a single oral dose of 5 mg of ^{14}C-NDMA to female rats the maximum rate of $^{14}C0_2$ production was 12.4% of the dose/hour, and that 48% of the dose could be recovered as $^{14}C0_2$ in the exhaled air in 7 hours and 5.7% as ^{14}C (total label) in a 24 hour urine sample.

2.6.4.3 Dermal Exposure

No studies were located regarding the excretion of NDMA following dermal exposure of humans or animals.

2.7 INTERACTIONS WITH OTHER CHEMICALS

NDMA is normally formed by bacteria in the human stomach and small intestine, but not in the large intestine (Archer et al 1982; Spiegelhalder and Preussmann 1985; Zeisel et al. 1988). Also, rats and guinea pigs have

been shown to make NDMA in their stomachs (Hashimoto et al. 1976; Omori et al. 1979). Small amounts of NDMA are formed in the saliva of humans; concentrations can vary from 4 to 10 pg/mL depending on pH and type of food in the mouth (Rao et al. 1982). NDMA formation in the saliva can be increased by chemicals such as chlorogenic acid, which is found in coffee, and decreased by a number of synthetic additives, as well as caffeic acid, tannic acid and ascorbic acid, which are found in coffee, tea, and citrus fruits, respectively.

Consumption of alcohol has been shown to have complicated effects on the toxicity of NDMA. Rats that received alcohol (ethanol or isopropanol) by gavage for 2 days before receiving NDMA had more liver damage with the alcohol than without it (Lorr et al. 1984; Maling et al. 1975). Increased levels of plasma glutamic pyruvate transaminase were monitored and used as a sign of liver damage. Another study showed that 4 weeks of ethanol pretreatment in rats worsened the effects on DNA repair that occurred following DNA alkylation induced by NDMA (Mufti et al. 1988). There is at least one other study in rats, however, that showed that 23 days of pretreatment with ethanol decreased the hepatotoxicity of NDMA (Gellert et al. 1980).

Other substances to which people are exposed have been shown to alter the toxic effects of NDMA in rats. Vitamin E and calcium channel blocking agents have been shown to decrease the hepatotoxicity associated with NDMA (Landon et al. 1986; Skaare and Nafstad 1978). Selenium increased the toxic effect of NDMA on the liver (Skaare and Nafstad 1978) and cadmium increased the carcinogenic effect of NDMA in the kidney (Wade et al. 1987). NDMA induced higher incidences of stomach cancer in rats fed diets low in zinc than in those fed normal diets (Ng et al. 1984). Rats fed diets low in copper developed more kidney tumors from NDMA than rats fed normal diets (Carlton and Price 1973). In contrast, rats given NDMA and cupric acetate had fewer tumors than rats given NDMA (Yamane et al. 1984). Although these data indicate that simultaneous administration of other chemicals may augment NDMA toxicity in animals, it not clear how these simultaneous exposures may occur in humans.

2.8 POPUIATIONS THAT ARE UNUSUALTaY SUSCEPTIBLE

People with chronic renal failure produce more NDMA in their small intestines due to increased levels of bacterial growth than normal people do (Lele et al. 1983). This increase in NDMA can be blocked by injections of ascorbic acid or antibiotics, but is potentiated by alcohol (Lele et al. 1987). People who consume alcohol may be unusually susceptible to NDMA for reasons discussed in Section 2.7.

2.9 ADEQUACY OF THE DATABASE

Section 104 (i) (5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the

Public Health Service) to assess whether adequate information on the health effects of NDMA is available. Where adequate information is not available, ATSDR, in cooperation with the National Toxicology Program (NTP), is required to assure the initiation of **a** program of research designed to determine these health effects (and techniques for developing methods to determine such health effects). The following discussion highlights the availability, or absence, of exposure and toxicity information applicable to human health assessment. A statement of the relevance of identified data needs is also included. In a separate effort, ATSDR, in collaboration with NTP and EPA, will prioritize data needs across chemicals that have been profiled.

2.9.1 Existing Information on Health Effects of N-Nitrosodimethylamine

Information regarding health effects of NDMA in humans is limited to case reports of fatalities due to hepatotoxicity following ingestion or inhalation. Health effects of NDMA in animals have been investigated in numerous oral studies and several inhalation and dermal studies. As indicated in Figure 2-4, animal oral data are available for lethality, systemic toxicity, immunological effects, neurological effects, developmental effects, reproductive effects, genotoxic effects and cancer. These data indicate that hepatotoxicity and cancer are the most prominent NDMA-related effects.

2.9.2 Data Needs

Single Dose Exposure. Information on lethality in rats following single oral doses, including two LD^{50} values, are available. Information on hepatic effects in rats due to single oral exposures are also available. Additional single dose oral studies with rats would provide more information on thresholds for lethality and hepatotoxicity, and on nonhepatic effects. Studies on species other than the rat would provide data on interspecies differences. Single-exposure inhalation experiments provide limited information on lethality in rats, mice and dogs, and dermal/ocular effects in rats; additional studies could corroborate these data as well as provide NOAELs. Single application dermal studies would provide information on lethality and skin and eye irritation.

Repeated Dose Exposure. Numerous repeated dose studies of intermediate duration have been conducted with rats, mice and other species. These studies provide extensive information on doses and treatment schedules that are lethal and hepatotoxic but do not adequately identify thresholds for these effects, particularly in species that may be more sensitive (e.g., mink). Additional repeated dose oral studies designed to examine tissues other than the liver could provide useful information on nonhepatic systemic effects of NDMA. Oral studies conducted over periods longer than 20-30 weeks may not be necessary as sufficient evidence indicates that cancer will be the predominant effect. Repeated exposure inhalation studies could provide

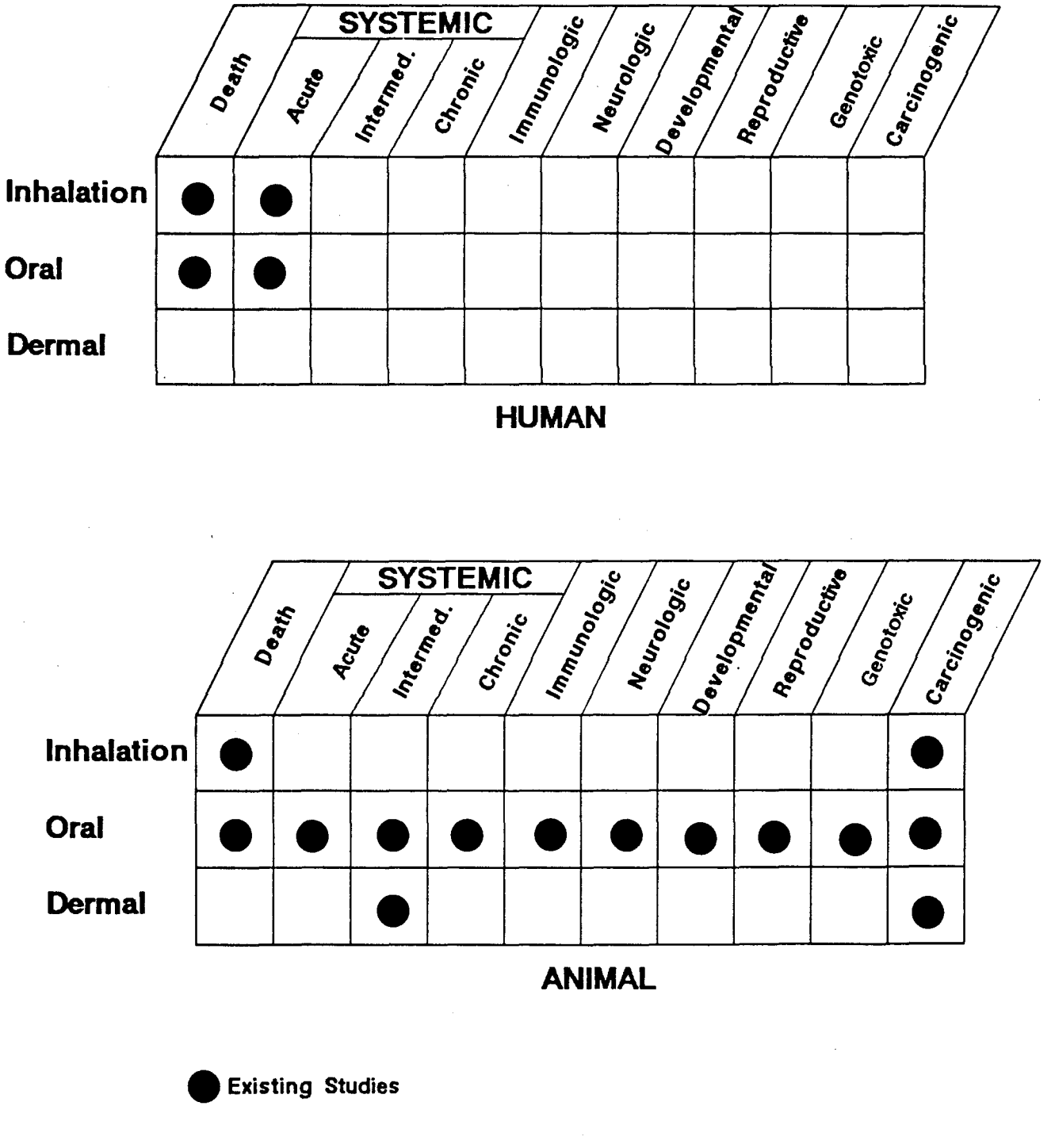

FIGURE 2-4. Existing Information on Health Effects of N-Nitrosodimethylamine

information on concentrations associated with lethality and systemic effects.

Chronic Exposure and Carcinogenicity. Chronic oral and inhalation studies of NDMA have been conducted with rats and mice. These studies indicate that the predomin&nt effect of chronic exposure to NDMA is cancer. As low doses of NDMA have been tested in chronic oral studies and it is established that intermediate duration exposure to NDMA is sufficient to induce cancer, additional chronic studies may not be needed.

Genotoxicity. The genotoxic potential of NDMA is established unequivocally. Only several studies, however, evaluated genotoxic effects in animals following oral or inhalation exposure to NDMA, and only several in vitro studies evaluated human cells. Additional studies, particularly assays with human cells and assays providing information on the potential for heritable mutations, would add to the data base on genotoxicity.

Reproductive Toxicity. Oral exposure to NDMA for 75 days prior to mating had no significant effect on time-to-conception in mice. Other reproductive indices or species have not been evaluated. Histological examinations of reproductive organs of animals exposed in subchronic and chronic studies would provide relevant data. Multigenerational or continuous breeding studies would provide further information regarding reproductive effects of NDMA in animals, which may be related to possible reproductive effects in human.

Developmental Toxicity. Evidence indicates that NDMA is fetotoxic to rats and mice, but NOAELs have not been defined. Well-conducted developmental studies using several exposure levels and environmentally relevant routes of exposure could provide the dose-response information necessary to determine the threshold for fetotoxicity and to determine the possible relevance and risk for humans. Additional studies also could determine if NDMA is a transplacental carcinogen.

Immunotoxicity. Information regarding immunological effects of NDMA in humans is not available. Immunosuppression by NDMA has been demonstrated in a number of intraperitoneal injection studies, but not in an oral study, with mice. Specific immunotoxicity tests or a battery of immunotoxicity tests in which NDMA is administered by the oral route would provide a better assessment of possible immunotoxic effects. Sensitization tests in animals could provide information on whether an allergic response to NDMA is likely in humans. Additional studies also could determine if NDMA is a transplacental carcinogen.

Neurotoxicity. Dogs that were orally treated with NDMA reportedly experienced central nervous system depression, but it is likely that this effect is a consequence of liver damage rather than direct neurotoxicity. Additional information pertaining to neurotoxicity was not found.

Neurotoxicity tests in animals exposed to NDMA could provide additional information on possible neurotoxic effects.

Epidemiological and Human Dosimetry Studies. The only information available concerning effects of NDMA in humans comes from cases of acute poisoning and subsequent death. In these cases, hemorrhagic and necrotic alterations and cirrhosis of the liver were observed. On the other hand, effects in animals have been well documented (Section 2.2). Attempts have been made to measure occupational exposure to NDMA, in particular in the rubber industry. Unfortunately these attempts have failed because NDMA is metabolized almost completely to CO_2 and water. Excretion rates for NDMA measured in experimental animals are in the order of 0.02% of the ingested dose (Spiegelhalder 1984). Although unchanged NDMA is unlikely to be detected in the urine, it may be possible to measure urinary excretion of nonspecific DNA adducts (e.g., 7-methylguanine). As stated by Spiegelhalder (1984), limited information is available on airborne exposures of individual workers for the following reasons: 1) usually workers are exposed to a variety of chemicals and there is cross-contamination between jobs, 2) transfers from job to job involve different exposures, 3) increases in cancer incidences most likely result from exposures that occurred in the past, when no exposure data were available, and 4) no comprehensive study has been conducted so far. Epidemiology studies of individuals who live in areas where NDMA has been detected are necessary to obtain information on whether NDMA induces effects in humans similar to those seen in animals.

Biomarkers of Disease. Since acute NDMA poisoning in humans caused severe liver disease, sensitive clinical biochemistry liver function tests might detect early hepatic damage from toxic exposure to NDMA. Recently, Wild et al. (1987), using a radioimmunoassay, were able to detect elevated levels of the promutagenic lesion O^6 -methyldeoxyguanosine in DNA of esophageal cells from individuals with high incidence of esophageal and stomach cancer. These individuals were found to consume foods with a relatively high content of nitrosamines.

Disease Registries. The only known health effects of NDMA on humans are those obtained from acute poisoning cases, in which postmortem examination revealed severe liver damage. If disease states attributed to exposure to NDMA could be identified by epidemiological studies, the number of individuals affected, the exposure levels involved, and the factors associated with identifying the disease in a given population, such as, the vicinity to hazardous waste sites or industrial plants, could be determined.

Bioavailability from Environmental Media. No studies were located regarding the bioavailability of NDMA from environmental media. Since NDMA has been detected in ambient air, water and soil (ppb levels), it is important to determine if NDMA can be absorbed by humans from environmental samples. It must be noted that NDMA has been found in trace amounts in some foods and beverages and that endogenous formation of NDMA has been found to occur from the nitrosation of amines in the gastrointestinal tract. An

understanding of the bioavailability of NDMA from environmental media may be obtained by studying the biological fluids of individuals exposed in the workplace or through the ingestion of NDMA-containing foods and beverages. The limited information available regarding absorption parameters of NDMA in experimental animals indicates that NDMA is rapidly absorbed from the gastrointestinal tract; therefore, one can assume that if water or soil contaminated with NDMA are ingested, NDMA will be readily absorbed.

Food Chain Bioaccumulation. No studies were available concerning food chain bioaccumulation of NDMA from environmental sources. NDMA has been detected in samples of cooked fish and meat. However, occurrence of NDMA in these samples is not the result of bioaccumulation but is the result of formation during preservation and/or cooking (Scanlan 1983). Estimation techniques have been used to determine that NDMA would not bioaccumulate in lipids of fish (see Section 5.3.1). Based on this limited amount of information, it is speculated that human exposure to NDMA through diet is not the result of food chain bioaccumulation. Monitoring for the accumulation of NDMA in organisms from several trophic levels could be used to support this conclusion.

Absorption, Distribution, Metabolism, Excretion. Examination of Section 2.6 clearly indicates that oral administration of NDMA has been the preferred route for studying its absorption, distribution, metabolism and excretion. This is not surprising since oral administration is easier to monitor when compared to other routes. The oral route seems to be the most pertinent to study since humans are most likely to be exposed to nitrosamines orally. Toxicokinetic data with regard to dermal and inhalation exposure of NDMA are clearly lacking. Furthermore, dermal and inhalation exposures may lead to different metabolic pathways and patterns of distribution and excretion, which could account for differences in the degree of toxicity exhibited by different routes of exposure. The metabolism of NDMA in isolated microsomal preparations seems to be well understood, but studies with cultured human cells could provide additional useful information. However, exploration of the denitrosation mechanism as an alternative to a-hydroxylation requires more attention. Determination of the urinary excretion of NDMA in control human volunteers and in individuals known to consume foods with high contents of nitrosamines could provide information concerning absorption and excretion of the xenobiotic.

Comparative Toxicokinetics. No studies were located regarding comparative toxicokinetics of NDMA in vivo. In vivo studies are available indicating differences in hepatic 0^6 -methylguanine repair activity among rodent species (O'Connor et al. 1982). A report by Prasanna et al. (1985) indicates that the in vitro metabolism of NDMA by liver microsomes from hamsters, rats and chickens is qualitatively similar, but with different rates. Montesano et al. (1982) showed that liver slices from humans have a metabolic capacity to activate NDMA similar to that found in rats and slightly lower than that found in liver slices from hamsters. Differences among species in the toxic responses to a chemical can be attributed to

differences in the toxicokinetic parameters. This seems to be particularly true for N-nitrosamines in general (Lijinsky 1987). The fact that a number of factors (animal species, route of exposure, dosing schedule) appear to determine the organ-specificity and the severity of the effect of NDMA indicates that caution must be exercised when assuming possible effects in humans. Although little information is available regarding the toxicokinetics of NDMA in humans, analysis of NDMA in the urine of individuals accidentally exposed to the chemical or of individuals consuming foods with a relatively high content of NDMA could provide quantitative information on absorption and excretion.

2.9.3 On-going Studies

Two studies regarding the immunotoxicity of NDMA are known to be ongoing (Federal Research In Progress, 1988). One is investigating the immunosuppressive activity of subchronic and chronic administration of NDMA, specifically the in vitro antibody response of NDMA treated spleen cell suspensions to a number of mutagens. This research is being performed by Holsapple at Virginia Commonwealth University. A second study, performed by Schook at the University of Illinois, is attempting to identify molecular mechanisms for the immunosuppressive effects of NDMA.

In research being conducted by Anderson at the Division of Cancer Etiology, National Cancer Institute, NDMA is being examined for its ability to cause neurogenic tumors in mice by transplacental exposure.

In studies sponsored by NIEHS, Faustman at the Univerity of Washington is evaluating NDMA and other related N-nitroso compounds for their in vitro developmental toxicity (Faustman 1989).

A number of ongoing studies are investigating the metabolism of NDMA (Federal Research in Progress, 1988). These include N-nitroso compound detoxification by Jensen at Temple University, Philadelphia, PA, formation and metabolism of nitrosamines in pigs by Magee at Temple University, metabolism and genotoxicity of nitrosamines in rats by Rogan at the University of Nebraska, Omaha, NE, and enzymology of nitrosamine metabolism in rats, mice and rabbits in a NCI-sponsored study by Yang at the University of Medicine and Dentistry, Newark, NJ. Other studies sponsored by NCI are being conducted to find means of shifting the balance of the metabolic pathway towards increasing inactivation and characterizing the possible role of α-nitrosamino radicals in the metabolism of NDMA (written communication, Keefer 1989).