CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Available toxicokinetic data pertaining to NDMA primarily consist of studies in animals exposed orally and via i.v. injection. No studies examining the absorption, distribution, metabolism, or excretion of NDMA after inhalation or dermal exposure in humans or animals were located. Quantitative data on NDMA kinetics are available from studies in rats, mice, patas monkeys, swine, beagles, hamsters, and ferrets.

- Absorption
  - Absorption of orally administered NDMA occurs primarily in the small intestine.
  - Oral absorption is rapid and complete in all species tested.
  - The oral bioavailability (the fraction of an oral dose that passes through the liver unchanged and enters systemic circulation) of NDMA may vary across species, with estimates ranging from about 10% in hamsters and rats to >90% in beagles at comparable administered doses (1–3 mg/kg). Oral bioavailability may also vary with dose.
  - Absorption of inhaled NDMA is inferred from human fatalities after inhalation and limited animal data.

- Distribution
  - In rats, hamsters, and pigs, unmetabolized NDMA passes freely between blood and tissues, with little to no accumulation in any given tissue.
  - *In vitro* studies using plasma from several species showed that NDMA does not bind plasma proteins.

- Metabolism
  - NDMA is metabolized by microsomal membrane-bound CYP2E1, to hydroxymethyl-nitrosamine. The latter is nonenzymatically converted to formaldehyde and the reactive methyldiazonium ion; additional metabolic products include methanol and a reactive methyl carbonium ion. Denitrosation of NDMA, yielding formaldehyde and monomethylamine, has also been demonstrated.
  - Metabolism of NDMA is saturable. In both swine and beagles, metabolism is saturated at an oral dose of 5 mg/kg.
  - Clearance of NDMA from blood is primarily via metabolism.

- Excretion
  - Very little unchanged NDMA is excreted in urine after oral exposure.
  - Methylamine is the primary urinary metabolite in rats exposed to NDMA orally.
  - Enterohepatic circulation of NDMA has been shown in pigs.
  - Enterosalivary circulation of NDMA has been demonstrated in beagles.

As discussed further in Section 5.6, NDMA is produced endogenously through both acid-catalyzed nitrosation of amine precursors (primarily in the stomach) and through biologically catalyzed nitrosation

in other tissues including the oral cavity, intestine, liver, blood, and bladder (Hrudey et al. 2013). Estimates of the amount of NDMA produced endogenously vary widely. Using three different methods and available literature on measured human NDMA blood levels, O^6-methylguanine DNA adducts, and urinary excretion levels, Hrudey et al. (2013) estimated the rate of endogenous production to be approximately 1 mg/day (equivalent to 0.014 mg/kg/day for a 70-kg adult). In a study of volunteers in which urinary NDMA was measured before and after consuming fish meals rich in amines along with the acceptable daily intake of nitrate, Vermeer et al. (1998) estimated endogenous production of NDMA to be 174 µg/day (about 0.0029 mg/kg/day). Krul et al. (2004) employed an *in vitro* model of the human gastrointestinal tract to estimate NDMA formation occurring with gradual intake of nitrate at a range of doses from 0.1 to 10 times the acceptable daily intake. The study authors estimated cumulative NDMA amounts of 1.3–422 µg when a rapid decrease in gastric pH was simulated and 1.8–42.7 µg when gastric pH was modeled at slow decrease.

3.1.1 Absorption

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 (CARB 1986) after inhalation exposure. Absorption is also indicated by reports of human deaths following inhalation of NDMA (Freund 1937; Hamilton and Hardy 1974).

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 rapid and essentially complete. In studies of beagles, swine, patas monkeys, rats, and ferrets exposed to oral doses between 0.15 and 5 mg/kg, the maximum concentration of NDMA in blood was reached within 30 minutes (Anderson et al. 1992b; Gombar et al. 1987, 1988, 1990; Streeter et al. 1990a, 1990b; see Table 3-1). Less than 2% of the labelled compound could be recovered from the gastrointestinal tract 15 minutes after oral administration of ^{14}C-NDMA to rats (Gomez et al. 1977).

Table 3-1. Maximum Blood Concentration and Time to Maximum in Animals Exposed to N-Nitrosodimethylamine (NDMA) by Oral Administration

			Unchanged NDMA	
Reference(s)	Species	Oral dose (mg/kg)	Cmax (ng/mL)	Tmax (minutes)
Mico et al. 1985	Rat	0.15	NR	~15[a]
Hinuma et al. 1990	Rat	0.20	174	5
Anderson et al. 1992b; Gombar et al. 1990	Patas monkey	1.0	205–210	25–30
Wishnok et al. 1987	Ferret	1.0	NR	30
Hino et al. 2000	Beagle	2.0	~800 [a]	~30 [a]
Gombar et al. 1987	Beagle	1.0	424	20
		5.0	2,677	25
Gombar et al. 1988	Swine	1.0	144	23
		5.0	2,217	23

[a]Approximate values were estimated by visual inspection of data presented graphically.

NR = not reported

In the rat, NDMA is absorbed much faster from the small intestine than from the stomach, in both isolated preparations (Heading et al. 1974) and *in vivo* (Hinuma et al. 1990; Pegg and Perry 1981). Ishiwata et al. (1978) reported that in guinea pigs exposed to NDMA directly to the ligated stomach or small intestine, NDMA was absorbed more rapidly (measured as disappearance from excised tissues) from the small intestine. The rate of disappearance from both tissues in the 20 minutes after exposure followed first-order kinetics (Ishiwata et al. 1978).

Oral bioavailability estimates for unchanged NDMA, obtained by comparing the area under the blood concentration-time curves (AUCs) after oral and i.v. administration, varied by species in studies using oral doses of 0.15–3 mg/kg. Relatively low fractional bioavailability (8–31%) was observed for rats and hamsters (Mico et al. 1985; Streeter et al. 1990a, 1990b); bioavailability in patas monkeys and swine was higher (49–67%; Gombar et al. 1988, 1990), and the highest values were obtained with beagles (93%; Gombar et al. 1987). Because NDMA is essentially completely absorbed from the gastrointestinal tract and does not bind plasma proteins (Gombar et al. 1987, 1988; Streeter et al. 1990a, 1990b) or associate with erythrocytes, the reasons for the wide variation in bioavailability are not fully understood. Little to no unchanged NDMA is excreted in urine or expired air (Anderson et al. 1992b; Magee 1956; Swann et al. 1984). The higher bioavailability in larger species has been suggested to result from significant extrahepatic metabolism (Gombar et al. 1990). This hypothesis is supported by observations of constant systemic clearance rates (normalized to body weight) despite large differences in hepatic extraction ratios

(Gombar et al. 1990). Both kidneys and lungs have been shown to exhibit NDMA demethylase activity and may contribute to extrahepatic metabolism; however, there are no estimates of the extent or rate of renal or pulmonary NDMA metabolism in larger mammals

There are few data with which to evaluate the dose-dependence of NDMA oral bioavailability. Mico et al. (1985) reported an oral bioavailability estimate of 21% in male rats given deuterated NDMA at a dose of 0.15 mg/kg; this estimate is comparable to the bioavailability estimate of 31% obtained in male rats given 1 mg/kg deuterated NDMA (Streeter et al. 1990a). In contrast, Harrington et al. (1990) observed dose-dependent hepatic extraction of NDMA in swine, with little unmetabolized NDMA reaching the bloodstream after oral doses of 0.1 mg/kg, while larger fractions escaped the liver unchanged at doses of 1 and 10 mg/kg.

No studies were located regarding the absorption of NDMA following dermal exposure of humans or animals. Indirect evidence indicating that NDMA may be absorbed through the skin of mice 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 eight animals to a cage and could have licked the NDMA from each other or inhaled this volatile compound. In an *in vitro* assay using excised human skin obtained at autopsy, Brain et al. (1995) reported a percutaneous flux of 11.32 μg/cm^2 and absorbed fraction of 2.57% over 48 hours after application of an infinite dose of NDMA in isopropyl myristate.

3.1.2 Distribution

In mice, hamsters, and pigs, unmetabolized NDMA was widely distributed throughout the body after i.v. injection, passing freely between blood and tissues (Gombar et al. 1988, 1990; Streeter et al. 1990b). In these species, the steady-state volume of distribution (V_{ss}) was approximately equal to total body water, suggesting little to no accumulation in tissues. In beagles, however, the V_{ss} (1.7–2.1 L/kg; Gombar et al. 1987; Hino et al. 2000) exceeded total body water (0.693 L/kg; Davies and Morris 1993), suggesting significant tissue accumulation. Table 3-2 shows V_{ss} values for several species. Gombar et al. (1990) used these data to derive an allometric equation for body weight scaling of V_{ss} and estimated a NDMA V_{ss} of 64,800 mL (~926 mL/kg) for a 70-kg human.

Table 3-2. Species Differences in Steady-State Volume of Distribution for Unmetabolized N-Nitrosodimethylamine (NDMA) (V_{ss}) after Intravenous Exposure

Reference(s)	Species	Intravenous dose(s) (mg/kg)	V_{ss} (mL/kg)
Gombar et al. 1990[a]	Mouse	1.0–2.0	769–796
Mico et al. 1985	Rat	0.10	297
Streeter et al. 1990b	Hamster	0.31	582
Hino et al. 2000; Gombar et al. 1987	Beagle	1–2	1,700–2,100
Gombar et al. 1988	Swine	0.1–1.0	1,000–1,900
Anderson et al. 1992b; Gombar et al. 1990[a]	Patas monkey	0.5–5.0	1,027–1,417

[a]Vd values from Gombar et al. (1990) were converted from mL to mL/kg using animal body weights reported by the study authors.

NDMA = N-nitrosodimethylamine; V_{ss} = steady-state volume of distribution

Unmetabolized NDMA was also observed 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 (Johansson and Tjalve 1978; Magee 1956). Wishnok et al. (1978) reported a similar finding in rats following i.p. injections.

In rats that were administered 0.2 mg/kg NDMA by i.v. injection, concentrations of unmetabolized NDMA in liver, spleen, kidney, lung, and brain were approximately 70% of the arterial blood concentrations and declined in parallel with blood concentrations to nondetectable levels within 4 hours after exposure, suggesting that these tissues do not accumulate NDMA in the rat. One hour after a dose of 6 mg ^{14}C-NDMA/kg was administered by i.p. injection to mice, the liver contained 2 times as much radioactivity as the kidney, spleen, and thymus (Johnson et al. 1987).

In vitro experiments (using equilibrium dialysis or a micropartitioning system) to evaluate whether ^{14}C-NDMA binds plasma proteins have shown no evidence for binding in plasma from rats (Streeter et al. 1990a), hamsters (Streeter et al. 1990b), swine (Gombar et al. 1988), and beagles (Gombar et al. 1987) at concentrations between 1 and 1,000 ng/mL.

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

No studies were located regarding the distribution of NDMA following oral exposure of humans. Few studies have measured tissue levels of unmetabolized NDMA or NDMA-derived radioactivity in animals. Anderson et al. (1986) measured NDMA in tissues of A/J or A/JCr mice exposed to 50 ppm NDMA in drinking water for 1–4 weeks. Concentrations of unchanged NDMA in kidney, lung, and brain were similar to those in the blood, while liver concentrations were lower. For example, after 4 weeks of exposure, concentrations in blood, kidney, lung, and brain were 65, 51, 38, and 32 ppb (respectively), while a concentration of 6 ppb was measured in the liver (Anderson et al. 1986). Coadministration of ethanol, a competitive inhibitor of CYP2E1, increased the concentrations of NDMA in blood and all tissues; in the group receiving 50 ppm NDMA with ethanol for 4 weeks, concentrations were 218, 64, 444, 182, and 72 ppb in blood, kidney, lung, brain, and liver, respectively.

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 in tissue levels reported in this study are likely due to the study authors' measurement of radioactivity (including metabolites); studies that measured unchanged NDMA (e.g., Anderson et al. 1986) showed little variation in tissue concentrations. Measurable amounts of NDMA were reported in blood, liver, kidney, lungs, and brain of mice exposed to 5 mg/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 (s.c.) dose of 12.5 mg/kg of the chemical (Althoff et al. 1977). NDMA and/or its metabolites is also distributed to breast milk; when nursing rats were given NDMA by gavage, O^6-methylguanine adducts or NDMA-derived radiolabel were detected in DNA from pup kidney and liver (Chhabra et al. 2000; Diaz Gomez et al. 1986).

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.

Maternal-fetal Transfer. NDMA can cross the placenta, leading to fetal exposure. After pregnant patas monkeys were exposed to 1.0 mg/kg NDMA, O^6-methylguanine adducts were detected in both placental DNA and DNA in the fetal liver (Chhabra et al. 1995). Using a dual recirculating human placental perfusion model, Annola et al. (2009) detected radioactivity in the fetal circulation after ^{14}C-NDMA exposure to the maternal circulation, indicating transplacental transfer. The study authors noted that transportation across the placenta likely occurred by passive diffusion, as the rate of transfer was similar

to that of antipyrine and the radioactivity levels in maternal and fetal circulations equilibrated within 3 hours (Annola et al. 2009). Co-treatment of perfused human placentas with ethanol and NDMA did not alter the placental transfer of NDMA (Veid et al. 2011).

3.1.3 Metabolism

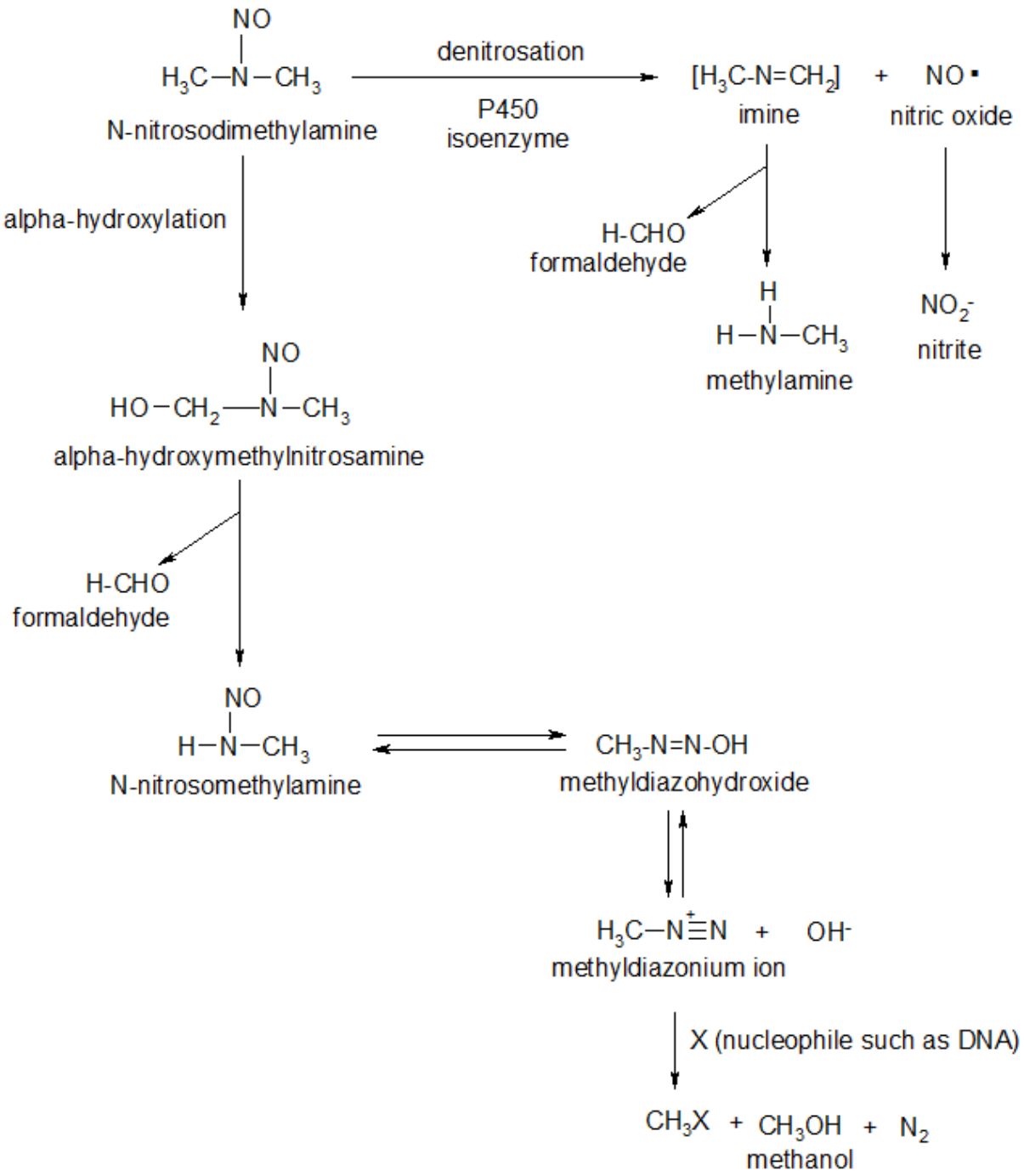
Metabolism of NDMA involves two pathways: α-hydroxylation or denitrosation. It is primarily the hydroxylation pathway that is believed to yield toxic metabolites responsible for liver toxicity and carcinogenicity (George et al. 2019; WHO 2008). Denitrosation of NDMA, yielding formaldehyde (an alkylating agent) and monomethylamine, has also been demonstrated. In rats exposed to doses of ~1 mg/kg NDMA orally, measurement of monomethylamine in blood showed that denitrosation accounted for approximately 21% of total NDMA elimination (Streeter et al. 1990a). Urinary excretion of labelled methylamine was also observed after i.v. administration of ^{14}C-NDMA to rats (Keefer et al. 1987), and methylamine was detected in human liver microsomes exposed to NDMA (Yoo et al. 1988).

α-Hydroxylation of NDMA is catalyzed by cytochrome p450 isozymes, forming α-hydroxymethyl-nitrosamine, which decomposes to monomethylnitrosamine and formaldehyde (George et al. 2019; WHO 2008). Monomethylnitrosamine is unstable and is non-enzymatically converted to formaldehyde and the reactive methyldiazonium ion. Formaldehyde is subsequently oxidized to carbon dioxide or reduced to form methanol. The methyldiazonium ion is an alkylating agent that methylates macromolecules including nucleic acids and proteins (Magee and Hultin 1962).

As discussed in Section 2.9, both formaldehyde and methanol are toxic to the liver and are believed to play a role in the hepatic effects of NDMA. However, the most toxic metabolite is believed to be the methyldiazonium ion. For example, *in vitro* experiments in rat hepatocytes exposed to metabolites of NDMA showed that monomethylamine, formaldehyde, and methanol did not produce cytotoxicity comparable to the parent compound, while a precursor of the methanediazonium ion produced cytotoxicity equivalent to that induced by NDMA (Lee et al. 1996).

The metabolism of NDMA is summarized in Figure 3-1.

Figure 3-1. Metabolism of N-Nitrosodimethylamine



Sources: George et al. 2019; Haggerty and Holsapple 1990; Keefer et al. 1987; Lee et al. 1996; Streeter et al. 1990a; Yoo et al. 1988

In vitro assays have shown that several CYP isozymes are involved in the α-hydroxylation of NDMA, but the enzyme that most efficiently catalyzes this reaction is CYP2E1 (Yang et al. 1985, 1990; Yoo et al. 1988, 1990; Sulc et al. 2004). Sulc et al. (2010) compared the kinetics of NDMA hydroxylation by purified CYP2B4, CYP3A6, and CYP2E1 isolated from rabbit liver after pretreatment with specific enzyme inducers. The lowest Km (Michales-Menten constant, 7.5 µmol/L) and highest Vmax (maximal reaction velocity, 3.8 nmol formaldehyde/minute/nmol CYP) were observed for CYP2E1, but both CYP2B4 (Km of 180 µmol/L; Vmax of 1.8 nmol formaldehyde/minute/nmol CYP) and CYP3A6 (Km of 30 µmol/L; Vmax of 1.3 nmol formaldehyde/minute/nmol CYP) were also active in hydroxylating NDMA (Sulc et al. 2010). Similar results were seen in liver microsomes from rabbits pretreated with ethanol or phenobarbital; microsomes pretreated with ethanol (increasing primarily CYP2E1 activity) exhibited the lower Km and higher Vmax compared with those pretreated with phenobarbital (increasing primarily CYP2B4 activity) (Sulc et al. 2004). Using pretreatments to inhibit enzymes of the CYP2A family, Pelkonen et al. (1994) observed only weak inhibition of NDMA metabolism in liver microsomes isolated from the pretreated hamsters, suggesting little to no role for these enzymes in its metabolism in hamsters.

Human liver microsomes have been shown to demethylate NDMA, with substantial interindividual variation in the extent of metabolism (Bellec et al. 1996; Camus et al. 1993). In genetically modified human cells stably expressing specific human P450s, CYP2E1 was also shown to be the primary isozyme involved in demethylation of NDMA (measured as production of formaldehyde) (Bellec et al. 1996). In this study, CYPs 1A2, 2A6, 2C8, 2C9, 2D6, and 3A4 were also shown to produce measurable formaldehyde, while CYPs 1A1 and 2C19 did not (Bellec et al. 1996).

Fujita and Kamataki (2001) tested the mutagenicity of NDMA in Ames tests using genetically modified *Salmonella typhimurium* strains expressing 11 different CYP enzymes. The investigators confirmed that metabolism by CYP2E1 yielded a mutagenic response to NDMA; none of the other enzymes did.

In vivo studies have indicated that metabolism of NDMA is saturable in swine and beagles. After oral exposure in both species, measurements of the area under the blood NDMA concentration:time curve were not proportional to dose suggesting saturation of metabolism at doses of about 5 mg/kg (Gombar et al. 1987, 1988). This finding is supported by evidence for competitive inhibition of NDMA metabolism by ethanol. When mice and patas monkeys were co-exposed to ethanol and NDMA by oral administration, greater quantities of NDMA escaped first-pass metabolism, presumably due to ethanol's competitive inhibition of CYP2E1 (Anderson et al. 1986, 1992b). Metabolic saturation is also supported

by evidence that different forms of enzymes appear to be responsible for NDMA metabolism at differing doses (Kroeger-Koepke and Michejda 1979; Lotlikar et al. 1978).

Studies of NDMA toxicokinetics in multiple species (rats, mice, hamsters, dogs, swine, and patas monkeys) exposed orally have shown that clearance of NDMA from blood is primarily via metabolism (Anderson et al. 1992b; Gombar et al. 1987, 1988, 1990; Hino et al. 2000; Streeter et al. 1990a, 1990b).

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

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

Hepatic extraction of NDMA was dose-dependent in pigs. After pigs were given NDMA orally at doses of 0.1, 1, or 10 mg/kg, the concentrations of unchanged NDMA were measured in hepatic portal blood (entering the liver from the gastrointestinal tract) and hepatic blood (exiting the liver) at various time points up to 10 hours after dosing (Harrington et al. 1987, 1990). At the highest dose, the maximum concentration of NDMA in hepatic blood was approximately half that of the concentration in portal blood. At lower doses, greater proportions of NDMA were metabolized, leading to smaller ratios of hepatic:portal blood concentration (about 1:4 at 1 mg/kg, and approaching 1:10 at 0.1 mg/kg, based on visual inspection of data presented graphically). Hepatic extraction was nearly complete at the lowest dose. These data suggest that the level of unchanged NDMA reaching the bloodstream is dependent on dose in pigs, and that at low doses (0.1 mg/kg), most NDMA is metabolized in the liver.

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

3.1.4 Excretion

NDMA was not detected (detection limit of 10 ng/L) in the urine of 59 nonsmokers who consumed drinking water containing 2 mg nitrate/L (geometric mean) (Levallois et al. 2000). Only one of the eight nitrosamines analyzed in the urine samples was detected: N-nitrosopiperidine (Levallois et al. 2000). Labelled CO_2 can be detected in the exhaled air 1 hour after i.p. administration of 5 mg/kg ^{14}C-NDMA to rats (Phillips et al. 1975). Hemminki (1982) administered labelled NDMA by i.p. 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 amino acids, fraction II was composed of allantoin and a metabolite of thiazolidine-4-carboxylic acid, and fraction III was composed of 7-methylguanine.

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 (CARB 1986).

Very little human data are available on the excretion of NDMA after oral exposure. Spiegelhalder et al. (1982) reported that in a 24-hour period, 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.

Only small amounts of unchanged NDMA were recovered in the urine of rats up to 24 hours after a single oral dose of 50 mg or i.v. dose of 500 mg/kg; the cumulative amounts excreted represented about 1.7% of the oral dose and 4.7–11% of the i.v. dose (Magee 1956). No NDMA was detected in feces samples over the same time frame (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. After i.v. administration of 1 mg/kg NDMA, rats excreted 0.11% of the dose as unchanged NDMA in urine (Streeter et al. 1990a). No unchanged NDMA was detected in urine of beagles in the 24 hours after i.v. dose of 1 mg/kg (Gombar et al. 1987) or in the urine of hamsters in 72 hours after an i.v. dose of 0.31 mg/kg (Streeter et al. 1990b). In patas monkeys given 1 mg/kg NDMA (Anderson et al. 1992b) and pigs given 10 mg/kg (Harrington et al. 1987, 1990) by i.v. administration, trace amounts of unchanged NDMA were detected in urine.

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 $^{14}CO_2$ production was 12.4% of the dose/hour, and that 48% of the dose could be recovered as $^{14}CO_2$ in the exhaled air in 7 hours and 5.7% as ^{14}C (total label) in a 24-hour urine sample. Excretion of monomethylamine resulting from NDMA denitrosation was demonstrated in rats given ^{14}C-NDMA intravenously (1 mg/kg). During the 72 hours after injection, 5.63% of the administered dose was excreted as monomethylamine (Streeter et al. 1990a). The authors estimated that monomethylamine accounted for as much as 21% of total NDMA elimination in rats.

Harrington et al. (1987, 1990) demonstrated that NDMA is secreted into the bile of pigs after intraarterial injection of a 10 mg/kg dose. Concentrations in bile reached blood levels within an hour after injection and peaked about 2 hours after injection. Biliary levels of NDMA declined at approximately the same rate as blood levels (Harrington et al. 1987, 1990). In rats exposed to NDMA by i.p. injection

(20 mg/kg), biliary excretion of NDMA accounted for 2.74–4.38% of the administered dose (Alaneme and Maduagwu 2004). Biliary excretion was lowest in rats given a very low protein diet (3.4%) and highest in those given a high protein diet (64%) (Alaneme and Maduagwu 2004).

Enterosalivary circulation of NDMA was observed in a study of beagle dogs (Hino et al. 2000). NDMA was detected in the dogs' saliva 15 minutes after oral or i.v. doses of 2 mg/kg NDMA, and salivary concentrations were comparable to or higher than plasma concentrations (Hino et al. 2000). After i.v. exposure, the concentration in salivary showed monoexponential decline similar to that seen in plasma. However, after oral exposure, plasma and saliva concentrations both remained relatively constant during the 2 hours following exposure when measurements were made, suggesting reabsorption from swallowed saliva. The study authors estimated that salivary excretion accounted for only about 2.4% of total body clearance of NDMA (Hino et al. 2000).

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

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

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

PBPK modeling studies for NDMA were not located in the literature reviewed.

3.1.6 Animal-to-Human Extrapolations

There are large interspecies differences in the systemic availability of unmetabolized NDMA, ranging from 8% in rats to 93% in beagles (based on AUC for unchanged NDMA in blood after oral and i.v. dosing; Gombar et al. 1987, 1988, 1990; Streeter et al. 1990a, 1990b). In patas monkeys, the only

nonhuman primate tested, systemic availability was 49%. Based on the systemic availability estimates, it has been suggested that in larger species, a significant portion of the NDMA dose escapes first-pass metabolism and is metabolized elsewhere (based on observation that systemic clearance rates normalized to body weight are similar across species despite differing hepatic extraction ratios) (Gombar et al. 1990). Because toxicity is induced by a metabolite, there may be other target organ(s) in larger species depending on where metabolism occurs. No data on other potential target organs in larger species are available, and epidemiological studies are not adequate to identify a target organ for oral exposure to NDMA in humans because they have focused on associations with cancer.

The primary CYP involved in demethylation of NDMA is CYP2E1 in both laboratory animals and in human liver extracts (see Section 3.1.3), demonstrating that humans are capable of NDMA bioactivation.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to NDMA are discussed in Section 5.7, Populations with Potentially High Exposures.

Data on NDMA levels measured in human infant blood or tissues have not been reported. Infants may be exposed to NDMA in infant formula, drinking water, food, and air (particularly in indoor environments with ambient tobacco smoke). Infants may also be exposed to very low levels of leaching from rubber baby bottle nipples or pacifiers; Sections 5.5 and 5.6 provide further information on these potential exposures. Two older studies (Lakritz and Pensabene 1984; Uibu et al. 1996) reported detections of

NDMA in human breast milk, but more recent data are not available. Studies of animals exposed during pregnancy demonstrate that NDMA crosses the placenta (Althoff et al. 1977; Chhabra et al. 1995) and can be excreted in breast milk (Chhabra et al. 2000; Diaz Gomez et al. 1986).

The susceptibility of infants and children to NDMA toxicity is complex, with some factors suggesting decreased susceptibility (e.g., reduced metabolic activation) and others suggesting increased susceptibility (e.g., reduced ability to repair DNA adducts).

Age-Related Pharmacokinetic Differences. Bioactivation of NDMA results from its oxidative metabolism, primarily via CYP2E1. The expression and activity of CYP2E1 varies by age, with lowest levels seen in infants. Vieira et al. (1996) evaluated CYP2E1 protein and ribonucleic acid (RNA) levels in hepatic microsomes from humans of various ages. The study authors observed no detectable CYP2E1 protein, and very little messenger RNA (mRNA), in hepatic microsomes from human fetuses. Within the first 24 hours after birth, CYP2E1 levels reached approximately 20% of adult activity; levels increased steadily over the first year of life, reaching about 80% of adult levels by 1 year of age (Vieira et al. 1996). Few differences in CYP2E1 activity are seen among children and adults. In a study of older children and adults, Blanco et al. (2000) observed no significant difference in CYP2E1 activity toward ethoxycoumarin in livers from humans <10, 10–60, or >60 years old.

Age-related differences in NDMA metabolic capacity have been seen in animals. No CYP2E1 protein was detected in livers from rat fetuses obtained at GD 10 or 20, but CYP2E1 was detectable in neonatal (4-day-old) rat liver (Borlakoglu et al. 1993). CYP2E1 mRNA levels did not differ with age. NDMA-demethylase activity was not detectable in fetal rat liver microsomes but increased more than 3-fold between PND 4 and 60 (Borlakoglu et al. 1993). In mice, hepatic NDMA-demethylase activity was present as early as GD 16 (3% of adult levels) and increased steadily after birth, reaching adult levels by PND 7 (Anderson et al. 2000; Jannetti and Anderson 1981). Yoo et al. (1987) observed increased NDMA-demethylase activity (and mutagenicity) in liver microsomes from weanling rats compared with adult rats; no age differences were seen in hamster liver microsomes.

Consumption of alcohol during pregnancy may increase the bioactivation of NDMA in infants. When pregnant rats were exposed to ethanol, hepatic CYP2E1 content was significantly increased in both maternal and fetal liver; the increase in the fetal liver was more than 2-fold compared with fetuses of rats that did not receive ethanol (Carpenter et al. 1997). Fetal liver microsomes from dams exposed to ethanol

also showed increased N-nitrosodimethylamine demethylase activity (1.5-fold higher compared with controls) (Carpenter et al. 1997)

Age-Related Differences in Susceptibility. Factors that may increase the susceptibility of infants and children (relative to adults) to the toxic effects of NDMA include increased cell proliferation associated with growth and lower capacity to repair DNA adducts, both of which may lead to greater mutation frequency in developing organisms. Coccia et al. (1988) observed markedly higher (>4-fold) levels of O^6-methylguanine adducts in newborn mice compared with adult mice after i.p. administration of the same dose of NDMA. These authors also measured the activity of O^6-methylguanine DNA methyltransferase (an enzyme that repairs DNA adducts induced by alkylating agents) and reported levels almost 4 times higher in adult mice compared with newborn mice, consistent with the differences in adduct levels (Coccia et al. 1988).

There is some evidence that younger animals may be more susceptible to NDMA mutagenicity. In one study, NDMA administration increased the mutation frequency in the livers of Big Blue (lacI) mice when administered as five daily doses of 2 mg/kg/day beginning at 3 weeks of age, but not when administered under the same conditions beginning at 6 weeks of age (reviewed by Lambert et al. 2005). The authors suggested that the difference in response could stem from age-related differences in metabolic activation, DNA adduct removal rates, or rates of mutation fixation. No difference in the fold-change in mutation frequency was observed in lac I transgenic mice exposed to a single oral dose of 10 mg/kg NDMA at 8–12 or 72 weeks of age (Tinwell et al. 1994a).

Transgenerational Effects. Available studies have not shown evidence for NDMA-induced germ cell mutagenicity or dominant lethal mutations (Doolittle et al. 1984; Epstein et al. 1972; Stott and Watanabe 1980); however, two studies suggested that NDMA may induce transplacental carcinogenesis after oral administration in rats (Aleksandrov 1974) or i.p. administration in mice (Anderson et al. 1989). Aleksandrov (1974) did not report data in control animals or specific tumor types, limiting the utility of this study. When pregnant C3H/HeNCr MTV- mice were treated by i.p. administration on GD 16 or 19, NDMA induced significant increases in hepatocellular carcinomas in male and female offspring and a significant increase in sarcomas in male offspring (Anderson et al. 1989). In contrast, Beebe et al. (1993) did not observe increases in lung or liver tumors in offspring of pregnant Swiss mice exposed by the same route at a higher dose on GD 19. Beebe et al. (1993) sacrificed the offspring at 1 year of age, while Anderson et al. (1989) did not sacrifice animals until they were moribund (average age 17–21 months), which may explain the disparate findings.

Other Factors Influencing Susceptibility. Because the liver is the primary target of NDMA toxicity, individuals with liver disease may be at increased risk from NDMA exposure. In addition, a recent study showed that infection of hamsters with cagA+ *H. pylori* or *Opisthorchis viverrine* (human liver fluke) prior to NDMA exposure resulted in increased cholangitis, hepatic lymphoid follicles, cholangiofibrosis, and cholangiocarcinoma incidence relative to NDMA alone (Dangtakot et al. 2021). Effects seen in the group infected with liver fluke were more severe than those seen in the group infected with *H. pylori.* While liver fluke infection is not common in the United States, *H. pylori* (all strains) infection is; Hooi et al. (2017) estimated the prevalence of infection in the United States to be 35.6%.

Interindividual variability in the expression and/or activity of CYP2E1 and other enzymes that bioactivate NDMA may lead to variable susceptibility to NDMA effects; however, there are no *in vivo* data in humans investigating this potential. Increased CYP2E1 activity has been demonstrated in obese individuals (Emery et al. 2003) and moderate to heavy consumers of alcohol (Liangpunsakul et al. 2005), suggesting a potential for greater bioactivation of NDMA in these individuals. Individuals consuming alcohol may be at greater risk of extrahepatic effects from NDMA exposure, based on studies of animals co-exposed to ethanol and NDMA via oral administration; these studies are discussed in Section 3.4. In animals, ethanol competitively inhibits the metabolic activation of NDMA in the liver, leading to greater systemic availability of unchanged NDMA and enhanced metabolic activation of NDMA in other tissues. Similar results may occur with other drugs that are metabolized by CYP2E1.

Polymorphisms in MGMT, the enzyme that repairs O^6-methylguanine adducts that are associated with cancer, may also alter the susceptibility of individuals to NDMA carcinogenicity. The importance of this enzyme in protecting against NDMA-induced cancers was shown in animals: MGMT knock-out mice exhibited higher incidences of lung and liver tumors compared with wild-type mice after i.p. exposure to NDMA (Iwakuma et al. 1997). Similarly, a recent study (Kay et al. 2021) showing that both the absence and the overexpression of *Aag* gene (encoding the alkyladenine DNA glycosylase) increase NDMA-induced effects (cancers and lethality, respectively) suggests that polymorphisms in the *Aag* gene may profoundly impact individual susceptibility to both cancer and other toxic effects of NDMA.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to NDMA are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for NDMA from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by NDMA are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Biomarkers of internal exposure to NDMA include urinary methylmercapturic acid and methylated DNA adducts. It should be noted that neither of these biomarkers distinguishes between exogenous and endogenously formed NDMA, and neither is specific to NDMA (other methylating agents will yield methylmercapturic acid and methylated DNA adducts).

As discussed in Section 3.1.2, metabolism of NDMA yields the alkylating methyldiazonium ion, which may be conjugated with glutathione and excreted as methylmercapturic acid. Recent advances in analytical techniques have enabled the detection of low levels of methylmercapturic acid. Scherer et al. (2010) developed a method using liquid chromatography (LC)-tandem mass spectrometry (MS/MS) analysis with positive electrospray ionization to measure methylmercapturic acid in urine along with other mercapturic acid products of tobacco-derived alkylating agents. Methylmercapturic acid was measured as a marker of exposure to all methylating agents, including NDMA as well as methyl halides and NNK (4-[methylnitrosamino]-1-[3-pyridyl]-1-butanone). The method was tested on urine from a group of 100 adult smokers of conventional cigarettes. In a clinic setting, these adults either continued smoking conventional cigarettes or were switched to an electronic cigarette or stopped smoking for 8 days and changes in the urinary levels of alkylated mercapturic acids were measured. While the levels of other tobacco-derived mercapturic acids (2-cyanoethylmercapturic acid and 2-hydroxyethylmercapturic acid) were substantially reduced in groups switching to electronic cigarettes or discontinuing smoking, urinary levels of methylmercapturic acid were not affected. The authors concluded that methylmercapturic acid was not a suitable biomarker for exposure to tobacco-derived methylating agents, speculating that endogenously produced methylating agents (such as NDMA and other endogenously produced nitrosamines) accounted for most of the methylmercapturic acid, masking the smaller contribution of tobacco-derived exposures (Scherer et al. 2010).

The methyldiazonium ion metabolite of NDMA also reacts with DNA to form methylated DNA adducts. Methylated DNA adducts are not specific to NDMA, as they may also occur as a result of exposure to other alkylating agents, including other nitrosamines that are endogenously produced (e.g., N-methyl-N-nitrosourea) or commonly encountered (N-nitroso-N-dimethylamine), as well as chemotherapeutic agents such as temozolomide and procarbazine. The primary methylated DNA adducts resulting from exposure to nitrosamines such as NDMA are, in order of declining prevalence, N^7-methylguanine, O^6-methylguanine, N^3-methyladenine, and O^4-methylthymine (Gallo et al. 2008). The O^6-methylguanine adduct is postulated to derive primarily from endogenous production of NDMA, and measurements in

humans have been used as one method to estimate endogenous production (Georgiadis et al. 2000; Hrudey et al. 2013). In a review examining the use of these adducts as biomarkers of nitrosamine exposure, Gallo et al. (2008) concluded that measurement of N^7- and O^6-methylguanine adducts in lymphocytes could be used as biomarkers for exogenous and endogenous nitrosamine exposure for the purpose of epidemiology studies. Immunoassay methods are recommended due to increased sensitivity and high throughput potential (Gallo et al. 2008; Georgiadis et al. 2010). It was noted, however, that these adducts are short-lived and may not represent long-term exposure (Gallo et al. 2008). Animal studies have demonstrated the presence of O^6-methylguanine adducts in liver (Souliotis et al. 1995, 2002), blood leukocytes (Kyrtopoulos 1998; Souliotis et al. 1995, 2002), and fetal tissues following oral exposure to NDMA (Chhabra et al. 1995). A discussion of the relevance of these DNA adducts to carcinogenesis is provided above in *Mechanisms* under Section 2.19 (Cancer).

3.3.2 Biomarkers of Effect

Methylated DNA adducts (described further as biomarkers of exposure in Section 3.3.1) may be considered biomarkers of preneoplastic changes induced by NDMA or other methylating agents. In particular, the O^6-methylguanine adduct induced by NDMA exposure is persistent and is known to induce mutations leading to tumors. Mutations (consisting of G:C to A:T transitions) derived from these adducts have been detected in lung tumors of mice exposed to NDMA and in transgenic mice exposed to NDMA (reviewed by WHO 2008).

NDMA has been used as a model for liver fibrosis and cancer in studies searching for biomarkers for these endpoints. Saha et al. (2007) evaluated the utility of several biomarkers for liver fibrosis in rats treated with NDMA by i.p. injection. These investigators observed significant correlations between the severity of liver histopathology and declining plasma protein C (an anti-inflammatory protein produced in the liver), C-reactive protein, haptoglobulin, albumin, and total protein. In addition, fibrosis severity was correlated with higher plasma levels of cytokines and chemokines including monocyte chemoattractant proteins 1 and 3 (MCP-1 and MCP-3) and macrophage-colony-stimulating-factor (M-CSF); increased circulating neutrophils; and elevated serum hyaluronic acid levels (Saha et al. 2007). George and Stern (2004) identified serum hyaluronan and hyaluronidase as early biomarkers of NDMA-induced hepatotoxicity. Both markers were markedly increased in the first 2 days of a 7-day exposure regimen; 2-fold increases in AST and ALT were first seen on day 3 (George and Stern 2004).

Kma and Sharan (2014) suggested that poly-ADP ribosylation (PAR, a post-translational modification of chromosomal proteins) of blood lymphocyte histones may represent a sensitive biomarker for cancer detection after observing time-dependent decline in PAR of specific histones in mice during exposure to carcinogenic doses of NDMA. The decline in PAR histones was correlated with changes in the superstructure (relaxation) of genomic DNA, making it more susceptible to degradation and, presumably, carcinogenicity. However, there is no indication that histone PAR levels are specific to NDMA.

3.4 INTERACTIONS WITH OTHER CHEMICALS

NDMA can be formed endogenously via acid-catalyzed nitrosation of amine precursors in the gastrointestinal tract, especially the stomach (Mirvish 1975). NDMA formation in the stomach has also been demonstrated in rats and guinea pigs treated with dimethylamino-containing drugs and sodium nitrite (Omori et al. 1979). Rao and co-authors (1982) detected small amounts of NDMA in human saliva incubated for 1 hour with aminopyrene or oxytetracycline at pH 3 or 4; concentrations ranged from 5 to 10 μg/mL (Rao et al. 1982). Addition of food constituents to the saliva generally inhibited the formation of NDMA. NDMA formation in the saliva was increased by chemicals such as chlorogenic acid, which is found in coffee, and decreased by caffeic acid, tannic acid, and ascorbic acid, which are found in coffee, tea, and citrus fruits, respectively.

Nutritional factors can influence NDMA-induced liver effects. Rats exposed to NDMA (in water) along with diets rich in proteins, cysteine, or choline exhibited less hepatic toxicity and greater regenerative activity compared to rats exposed to NDMA with a standard diet (Khanna and Puri 1966). In rats given NDMA with diets deficient in proteins, cysteine, or choline, hepatotoxicity was prolonged: there was minimal regenerative activity after 12 weeks, while the group receiving NDMA with the standard diet showed marked regenerative activity and pseudolobule formation at this time. Some of the liver effects were attributable to the nutritional deficiencies. Animals fed diets low in protein or cysteine without NDMA developed vascular congestion and necrosis after 12 weeks, and those on choline-deficient feed developed fatty metamorphosis and central vein congestion after 4 weeks (Khanna and Puri 1966).

Alcohol has been shown to competitively inhibit NDMA metabolic activation via CYP2E1, leading to mitigation of liver effects but potentiation of extrahepatic tumorigenicity in animals exposed to NDMA orally. In mice co-exposed to 50 ppm NDMA and 10–30% ethanol in drinking water, blood and tissue levels of NDMA were higher (≥10-fold in some cases) than in mice exposed only to NDMA, reflecting decreased metabolism (Anderson et al. 1986). Liver hemorrhage and necrosis were less severe in mice

co-treated with 10% ethanol compared with 50 ppm NDMA alone (Anderson et al. 1986). In contrast, when NDMA was administered by i.p. injection in rats pretreated or co-treated with alcohol (ethanol or isopropanol), the effects on the liver were more severe with the alcohol than without it (Lorr et al. 1984; Ma et al. 1991; Maling et al. 1975), presumably due to induction of CYP2E1 and enhanced metabolic activation of NDMA. In humans, moderate to heavy consumption of alcohol increases hepatic CYP2E1 activity (Liangpunsakul et al. 2005), which may increase the bioactivation of NDMA and its toxicity in these individuals.

Ethanol enhanced the tumorigenic effect of NDMA in the lungs and kidneys when both were administered orally in mice, either as a single dose or for 16–72 weeks via drinking water (Anderson 1988; Anderson et al. 1992a). When NDMA was administered by other routes (intravenous, intraperitoneal, or subcutaneous injection), ethanol did not influence tumor incidence or counts; thus, the increase in tumorigenesis was attributable to inhibition of first-pass clearance in the liver, enabling greater quantities of NDMA to circulate to other organs and tissues. Toxicokinetic studies in patas monkeys demonstrated a similar effect of ethanol. In this species, oral administration of NDMA and ethanol resulted in 10–50-fold increases in the area under the blood concentration:time curve and 4–13-fold increases in mean residence time compared with oral administration of NDMA alone (Anderson et al. 1992b). In rats, oral intake of alcohol and NDMA during lactation increased the formation of O^6-methylguanine adducts in offspring kidney and lung, while decreasing adducts in offspring liver (Chhabra et al. 2000). These studies demonstrate that in both laboratory rodents and primates, ethanol increases the systemic availability of orally-administered NDMA and the potential for extrahepatic toxicity, mutagenicity, or carcinogenicity. It is likely that other drugs or chemical that are metabolized by CYP2E1 may have similar effects.

Interactions between NDMA and heavy metals may vary depending on the valence state, compound, dose, route and timing of administration, and potentially the health effect of interest, as heavy metals can both induce and deplete levels of various antioxidants. Hexavalent chromium coexposure resulted in the increased formation of O^6-methylguanine DNA adducts in the livers of rats exposed to NDMA in drinking water (compared with NDMA alone) and decreased hepatic GSH levels (Ma et al. 2015). Pretreatment of rats with cadmium or zinc to induce metallothionein (a scavenger of reactive oxygen species [ROS]) before NDMA exposure resulted in increased levels of GSH and reductions in markers of toxicity including methemoglobin and nitric oxides (Rana and Kumar 2000, 2001). Consistent with a protective effect of zinc, NDMA induced higher incidences of stomach cancer in rats fed diets low in zinc than in those fed normal diets (Ng et al. 1984). However, Wade et al. (1987) observed synergistic effects

of cadmium and NDMA on kidney tumor incidence in rats when cadmium was administered after NDMA. Selenium pretreatment before NDMA exposure increased plasma AST levels without affecting the severity of hepatic necrosis, while decreasing plasma and liver concentrations of vitamin E (Skaare and Nafstad 1978). 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).

Subcutaneous administration of aminoacetonitrile (200 mg/kg) to female Wistar rats treated at the same time with 30 mg/kg NDMA (i.p.) decreased the metabolism of NDMA (as measured by clearance from the blood) as well as its methylation of nucleic acids in the liver and kidney (Fiume et al. 1970).

Klein et al. (1989) examined the influence of SO_2 and NO_X on NDMA-induced carcinogenicity in a long-term study of rats exposed by inhalation. The authors characterized their publication as an interim report, but a final report was not located in the published literature. Comparisons between groups treated with NDMA alone (0.2 ppm) or co-treated with 6 ppm of SO_2 or NO_X did not show any significant differences in body weight (data not reported) or incidences of nasal tumors after 20 months of exposure (Klein et al. 1989). Mortality was slightly higher in the group co-treated with NO_X and NDMA compared with NDMA alone (9/36 versus 4/36) but the difference was not statistically significant.

A number of studies have shown that liver fibrosis and cirrhosis induced by NDMA can alter the pharmacokinetics of drugs in laboratory animals. A review of such interactions by Lee and Oh (2015) reported effects of NDMA-induced cirrhosis in rats on plasma protein binding and clearance of drugs with a wide range of hepatic extraction ratios, including oltipraz (used to treat schistosomiasis), chlorzoxazone (a muscle relaxant), sildenafil (used to treat erectile dysfunction), omeprazole (a proton pump inhibitor used for gastric reflux), and DL-Praeruptorin A (a calcium influx blocker). In general, cirrhotic rats exhibited lower protein-binding and lower non-renal clearance rates for these medications. The alterations in non-renal clearance observed in the NDMA-treated rats were attributed to changes in hepatic intrinsic clearance due to changes in CYP expression in the liver, the fraction of free (unbound) drug in plasma, and hepatic blood flow. In cirrhotic rats, for example, expression of CYP2B1/2, CYP2C11, CYP2E1, and the CYP1A, CYP2D, and CYP3A subfamilies were significantly decreased compared with controls (Lee and Oh 2015). Effects on drug clearance were also noted in dogs with liver injury induced by oral administration of NDMA: clearance of antipyrine (a non-narcotic analgesic) and caffeine decreased with progressive liver disease (Boothe et al. 1994).

Numerous studies have been conducted to identify drugs, nutrients, and/or supplements that could be used to treat human liver fibrosis or cirrhosis using NDMA administration in rats as an animal model. In these studies, NDMA was administered by i.p. injection; thus, their relevance to human exposure to NDMA is limited. Many of these studies showed that coadministration of antioxidants can mitigate the hepatotoxicity of NDMA, which is consistent with oxidative stress as one mechanism by which NDMA induces liver injury (see Section 2.9). Some example antioxidants shown to modulate NDMA liver toxicity include resveratrol (Abdu and Al-Bogami 2019; Hong et al. 2010), gallic acid (Chen et al. 2018), silymarin and curcumin (George et al. 2006), hesperidin (Elshazly and Mahmoud 2014), and vitamin E (Skaare and Nafstad 1978). Other compounds that may mitigate liver effects of NDMA include those that decrease the activity of enzymes that metabolically activate NDMA. Examples include the adrenergic antagonist dibenamine (Stripp et al. 1974), aminoacetonitrile (Fiume et al. 1970), and dimethylformamide (Heath 1962).

Little information is available to evaluate potential synergistic or antagonistic effects of NDMA and other chemicals on noncarcinogenic effects on organs other than the liver. Administration of four daily oral doses of 3.75 mg/kg/day NDMA increased the sedative effects of pentobarbital; sleeping time increased 39% over that of control mice (Nishie et al. 1972).