CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of N-nitrosodi-n-propylamine. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

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

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. Animal oral studies are presented in Table 2-1 and Figure 2-2; no inhalation or dermal data were identified for N-nitrosodi-n-propylamine. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant

dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of N-nitrosodi-n-propylamine are indicated in Table 2-1 and Figure 2-2.

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

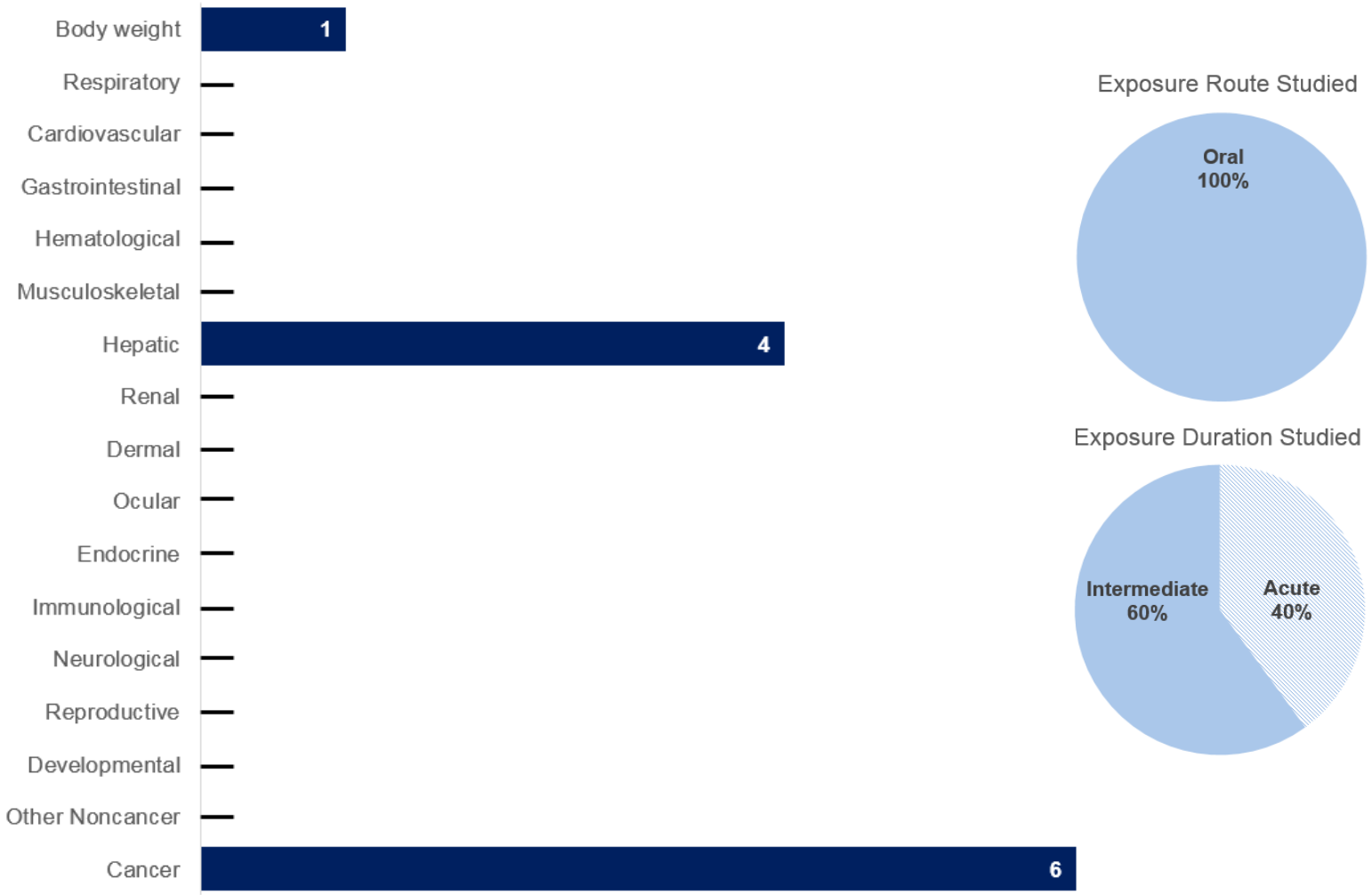
The health effects of N-nitrosodi-n-propylamine have been evaluated in laboratory animal studies. As illustrated in Figure 2-1, all of the health effects data come from oral exposure studies in animals. Animal data are only available for hepatic effects following acute exposure and cancer following intermediate-duration exposure.

The available animal studies suggest the following targets of toxicity:

- **Liver Endpoint:** Evidence of liver damage including increases in pentobarbital sleep time and necrosis have been observed in laboratory animals following acute-duration exposure.
- **Cancer Endpoint:** Forestomach, nasal, lung, and liver tumors have been observed in laboratory animals following intermediate-duration oral exposure.

Figure 2-1. Overview of the Number of Studies Examining N-Nitrosodi-n-Propylamine Health Effects*

Studies examined the potential hepatic or cancer effects of N-nitrosodi-n-propylamine
All studies evaluated health effects in **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 10 studies, including those finding no effect, have examined toxicity; some studies examined more than 1 endpoint.

Table 2-1. Levels of Significant Exposure to N-Nitrosodi-n-Propylamine – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
1	Rat (BD) NS	Once (G)	NR	LE, HP	Death			480	LD_{50}
					Hepatic			480	Necrosis
Druckery et al. 1967									
2	Mouse (Swiss Webster) 10 M	4 days (GO)	0, 40	FX	Hepatic		40		Hepatocellular swelling and increased pentobarbital sleep time
Nishie et al. 1972									
3	Rat (CD) 5 M	14 days (GW)	0, 10, 20, 40	CS, BW, OW, HP	Bd wt	20	40		13% decrease in body weight gain
					Hepatic		10		Minimal single cell hepatocellular necrosis at 10 mg/kg/day; decreased liver weight, minimal hepatocellular hypertrophy, mild centrilobular hepatocellular necrosis, minimal centrilobular inflammation, diffuse hepatocellular vacuolation at ≥20 mg/kg/day
Terashima et al. 2015									
4	Mouse (BALB/c) 6 F	1 week (F)		BC	Hepatic	9.5			Only assessed serum enzymes
Tyndall et al. 1978									
INTERMEDIATE EXPOSURE									
5	Rat (BD) 14-16NS	Lifetime (F)	0, 4, 8, 15, 30	HP	Cancer			4	Liver carcinoma
Druckery et al. 1967									

Table 2-1. Levels of Significant Exposure to N-Nitrosodi-n-Propylamine – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
6	Mouse (C57BL) 38M, 32F	2 days/week 50 weeks (G)	0,1	CS, BW, GN, HP	Cancer			1	Forestomach, pulmonary tumors
Griciute et al. 1982									
7	Rat (Fischer 344) 20 NR	5 days/week 30 weeks (W)	2.6	LE, HP	Death			2.6	8/20 deaths at week 30; 100% mortality 10 weeks post-exposure
					Cancer			2.6	Esophagus, forestomach tumors
Lijinsky and Reuber 1981									
8	Rat (Fischer 344) 12 F	2 days/week 30 weeks (GO)	6.3	LE, HP	Death			6.3	5/12 deaths 20-weeks post-exposure, 100% mortality 40 weeks post-exposure
					Cancer			6.3	Hepatic and nasal carcinomas
Lijinsky and Reuber 1983									
9	Rat (Fischer 344) 20 M	2 days/week 30 weeks (GO)	12.6	LE, HP	Death			12.6	100% mortality 10 weeks post-exposure
					Cancer			12.6	Hepatic, esophageal, lung, and nasal carcinomas
Lijinsky and Reuber 1983									
10	Rat (Sprague-Dawley) 15 M	5 days/week 30 weeks (W)	5.1	LE, HP	Death			5.1	100% mortality 30 weeks-post-exposure
					Cancer			5.1	Hepatic and esophageal carcinomas and nasal adenocarcinomas
Lijinsky and Taylor 1978, 1979									

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

BW = body weight; CS = clinical signs; (F) = exposure in feed; F = female(s); FX = fetal toxicity; (G) = gavage; (GO) = gavage in oil vehicle; GN = gross necropsy; HP = histopathology; LD_{50} = lethal dose, 50% mortality; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NR = not reported; (W) = water

Figure 2-2. Levels of Significant Exposure to N-Nitrosodi-n-Propylamine – Oral

Acute (≤ 14 days)

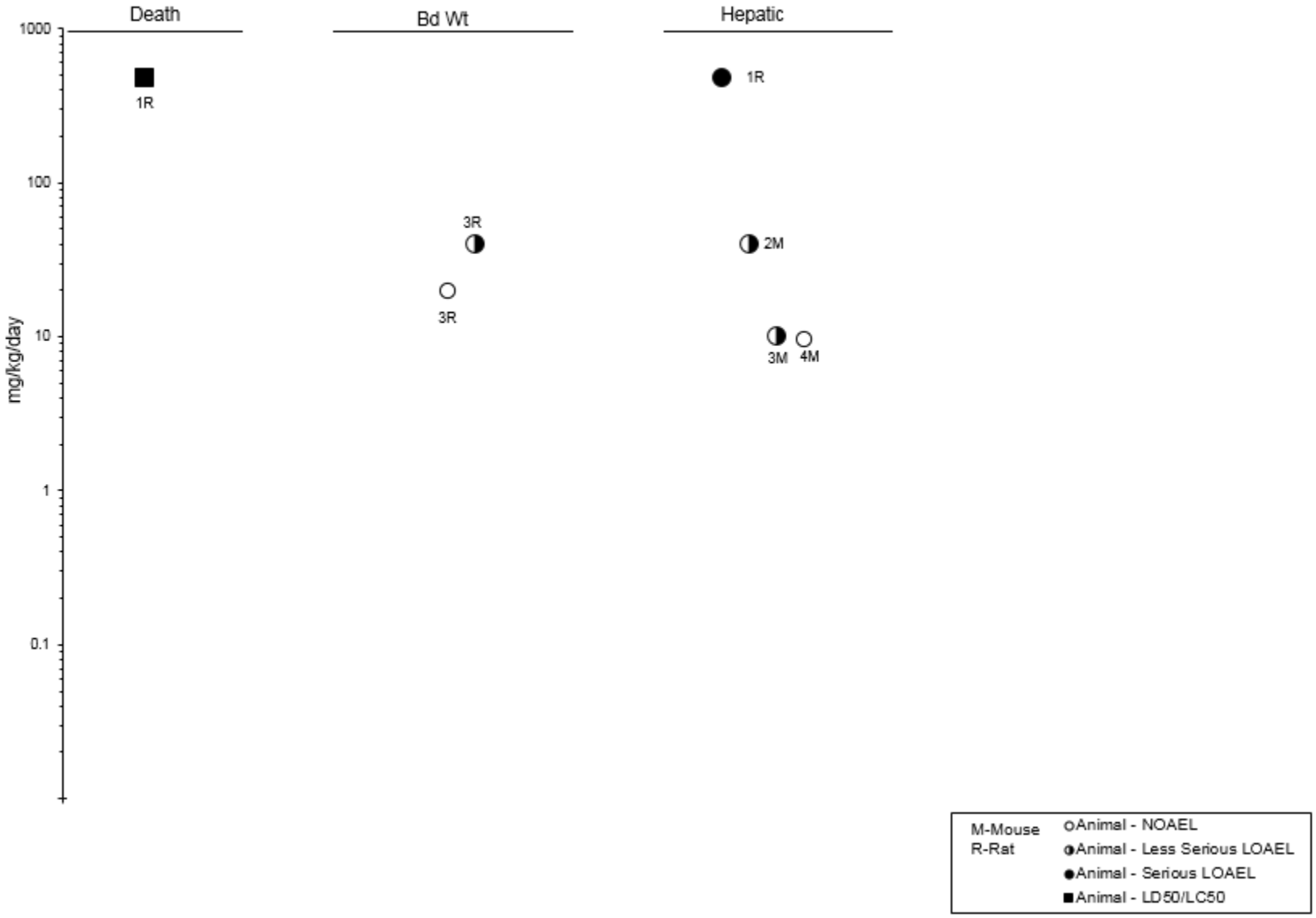
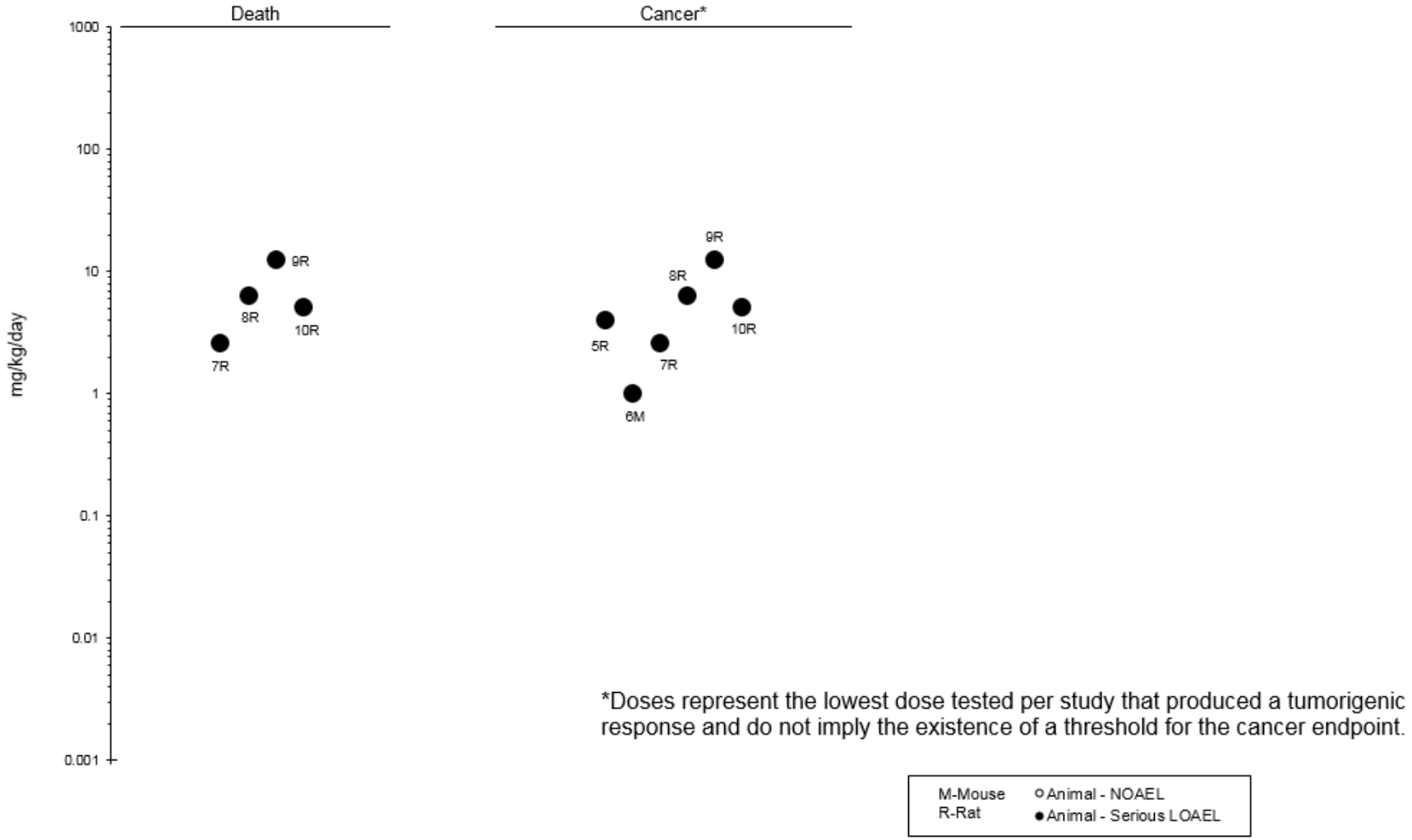


Figure 2-2. Levels of Significant Exposure to N-Nitrosodi-n-Propylamine – Oral
Intermediate (15-364 days)



2.2 DEATH

Druckrey et al. (1967) determined a single dose gavage LD_{50} of 480 mg/kg for N-nitrosodi-n-propylamine in rats. The value was determined using an unspecified graphic technique, but specific mortality data were not reported. Deaths occurred after 3–7 days and appear to have been due primarily to hepatotoxicity. Other acute oral lethality data were not located in the reviewed literature.

Decreased longevity occurred in rats that were treated with N-nitrosodi-n-propylamine at doses of 6.3 mg/kg/day (females) or 12.6 mg/kg/day (males) by gavage for 2 days/week for 30 weeks (Lijinsky and Reuber 1983), or 5.1 mg/kg/day (males) via drinking water for 5 days/week for 30 weeks (Lijinsky and Taylor 1978, 1979). Mortality in the Lijinsky and Reuber (1983) study was 92–100% after 40–60 weeks compared to 5–10% after 100 weeks in controls; comparable data were reported by Lijinsky and Taylor (1978, 1979) for the treated rats, but a control group was not used. The mortality in these studies was due to tumor development (see Section 2.19). No studies were located regarding survival in animals following chronic oral exposure to N-nitrosodi-n-propylamine.

2.3 BODY WEIGHT

Data on the effect of N-nitrosodi-n-propylamine on body weight are limited to an acute-duration oral study, which reported a 13% decrease in body weight gain in rats administered 40 mg/kg/day for 14 days (Terashima et al. 2015).

2.4 RESPIRATORY

No studies were located regarding respiratory effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine.

2.5 CARDIOVASCULAR

No studies were located regarding cardiovascular effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine.

2.6 GASTROINTESTINAL

No studies were located regarding gastrointestinal effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine.

2.7 HEMATOLOGICAL

No studies were located regarding hematological effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine.

2.8 MUSCULOSKELETAL

No studies were located regarding musculoskeletal effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine.

2.9 HEPATIC

Pathologic examinations of rats that received single lethal doses of various nitrosamines, including N-nitrosodi-n-propylamine, showed centrilobular necrosis and fatty degeneration of the liver (Druckrey et al. 1967). Specific doses of N-nitrosodi-n-propylamine that produced these effects were not reported, but the LD_{50} was determined to be 480 mg/kg; this dose is indicated in Table 2-1 and Figure 2-1 as a serious LOAEL for hepatic effects in rats due to acute oral exposure.

Nishie et al. (1972) determined pentobarbital sleeping time (PST) in mice that were treated by gavage with single doses or with four consecutive daily doses of various nitrosamines, including N-nitrosodi-n-propylamine. Doses of N-nitrosodi-n-propylamine were 160 mg/kg/day in the single-dose study and 40 mg/kg/day in the 4-day study. N-Nitrosodi-n-propylamine treatment resulted in significantly prolonged PST in both studies. Liver histology was evaluated in the 4-day study, but results of the histologic examinations were not reported specifically for any of the nitrosamines. Hepatic histological alterations attributed to unspecified nitrosamines included hepatocyte swelling and necrosis in the centrilobular areas; due to the inadequately reported data, it cannot be determined whether N-nitrosodi-n-propylamine was among the nitrosamines that produced these effects. However, considering the aforementioned findings for nitrosamines in general as well as evidence for hepatotoxicity of N-nitrosodi-n-propylamine and other nitrosamines from other studies, the increase in PST provides an indirect indication of adverse liver effects. Therefore, since N-nitrosodi-n-propylamine markedly increased PST in the 4-day study, 40 mg/kg/day can be regarded as a LOAEL for less serious hepatic effects due to acute oral exposure (Table 2-1 and Figure 2-1).

Liver histology and activities of liver-associated serum enzymes (aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, γ-glutamyl transferase) were unaltered in mice exposed to 9.5 mg/kg/day via drinking water for 1 week (Tyndall et al. 1978). In a 14-day gavage study, single cell hepatocellular necrosis was observed in rats administered 10 mg/kg/day (Terashima et al. 2015). Hepatocellular hypertrophy, necrosis, and vacuolation and centrilobular inflammation were observed in rats administered 20 and 40 mg/kg/day. The severity of the hepatocellular necrosis was graded as minimal at 10 mg/kg/day, mild at 20 mg/kg/day, and moderate at 40 mg/kg/day; the other lesions were graded as minimal.

In addition to these oral studies, increases in organ weight and generalized mild hydropic degeneration were observed in the livers of mice administered ≥50 mg/kg/day N-nitrosodi-n-propylamine via intraperitoneal injections for 7 days (Kaminski et al. 1989). Mild chronic hepatitis was also observed at 90 mg/kg/day.

2.10 RENAL

No studies were located regarding renal effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine.

2.11 DERMAL

No studies were located regarding dermal effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine.

2.12 OCULAR

No studies were located regarding ocular effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine.

2.13 ENDOCRINE

No studies were located regarding endocrine effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine.

2.14 IMMUNOLOGICAL

No studies were located regarding immunological effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine. A 7-day intraperitoneal injection study reported a suppressed antibody response to sheep red blood cells in mice administered ≥50 mg/kg/day; the ED_{50} was 60.8 mg/kg/day (Kaminiski et al. 1989). Decreases in spleen and thymus weights were also observed at ≥50 mg/kg/day.

2.15 NEUROLOGICAL

No studies were located regarding neurological effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine.

2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine.

2.17 DEVELOPMENTAL

No studies were located regarding developmental effects in humans or animals following inhalation, oral, or dermal exposure to N-nitrosodi-n-propylamine. Limited information regarding developmental effects of N-nitrosodi-n-propylamine is available from subcutaneous injection transplacental carcinogenesis studies with hamsters (Althoff and Grandjean 1979; Althoff et al. 1977a). Injection of a single dose of 100 mg N-nitrosodi-n-propylamine/kg on day 8, 10, 12, or 14 of gestation did not produce gross malformations in the offspring, but the scope of the examination was not specified. However, transplacental carcinogenicity was observed in the offspring of dams treated with N-nitrosodi-n-propylamine. There were no treatment-related effects on litter size, but postnatal mortality in the first 4 weeks was increased (Althoff et al. 1977a). Transplacental transport of N-nitrosodi-n-propylamine by the hamsters was demonstrated by detection of the chemical in the placenta, fetus, and amniotic fluid.

2.18 OTHER NONCANCER

Plasma esterase profiles were examined in mice exposed to various carcinogenic, weakly carcinogenic, and noncarcinogenic chemicals in the drinking water for 1 week (Tyndall et al. 1978). N-Nitrosodi-n-propylamine, administered at a dose of 9.5 mg/kg/day, produced esterase alterations that were similar to

those produced by other N-nitrosodialkylamines. The alterations were not accompanied by weight loss, altered liver-associated serum enzymes, or histologic effects. This study was conducted to determine whether altered esterase patterns in plasma would provide a more sensitive indicator of exposure to a carcinogenic chemical than standard clinical chemistry tests. It was concluded that it is not known if the altered esterase profiles that were observed for N-nitrosodi-n-propylamine, and the other carcinogens are related to carcinogenicity, toxicity, or metabolism. Since the biological significance of the altered esterase profiles is unknown, it cannot be determined if 9.5 mg/kg/day represents a NOAEL or LOAEL for serum chemistry alterations due to acute oral exposure.

2.19 CANCER

No studies involving exposure to airborne N-nitrosodi-n-propylamine were located. In an intratracheal instillation study (1.5 mg instilled once a week for 15 weeks), tracheal tumors were observed in 72% of hamsters (Ishinishi et al. 1988); no liver tumors were observed.

The carcinogenicity of N-nitrosodi-n-propylamine has been demonstrated in oral studies. High incidences of liver carcinomas, nasal cavity carcinomas, esophageal carcinomas and papillomas, forestomach tumors, or tongue tumors occurred in rats that were exposed to N-nitrosodi-n-propylamine by gavage at doses of 6.3 or 12.6 mg/kg/day for 2 days/week for 30 weeks (Lijinsky and Reuber 1983), via drinking water at a dose of 2.6 mg/kg/day for 5 days/week for 30 weeks (Lijinsky and Reuber 1981), via drinking water at a dose of 5.1 mg/kg/day for 5 days/week for 30 weeks (Lijinsky and Reuber 1981; Lijinsky and Taylor 1978, 1979), and via diet daily at reported doses of 4–30 mg/kg/day for life (survival duration not specified) (Druckrey et al. 1967). Tumor incidences in the liver, nasal cavity, esophagus, and forestomach were generally in the range of 60–l00%, and tongue tumor incidences ranged from 30 to 40%. The Lijinsky and Reuber (1983) study was the only study that used control groups; no tumors occurred in the control rats at any of the sites in which tumors developed in the treated rats. The lack of controls in the other studies is not considered to be a serious deficiency due to the high tumor incidences. As indicated in Section 2.2, tumor development in all of the rat studies was life-shortening.

In an oral carcinogenicity study conducted with mice, the animals received an estimated N-nitrosodi-n-propylamine dose of 1 mg/kg by gavage, twice a week for 50 weeks (Griciute et al. 1982). Incidences of forestomach papillomas, forestomach carcinomas, and pulmonary adenomas were significantly higher than in mice that were similarly treated with 40% ethanol; a vehicle (water) control was not used.

Additional support for the carcinogenicity of N-nitrosodi-n-propylamine comes from several parenteral administration studies. Weekly subcutaneous injections of N-nitrosodi-n-propylamine to rats (Althoff et al. 1973b, Reznik et al. 1975), mice (Dickhaus et al. 1977), and hamsters (Althoff et al. 1973a, 1977b; Pour et al. 1973, 1974) for life produced high incidences of tumors, primarily in the nasal cavity and other parts of the respiratory system, but also in the liver and esophagus. Subcutaneous injection of single 100 mg/kg doses of N-nitrosodi-n-propylamine into hamsters during gestation induced tumors, primarily in the respiratory and digestive tracts, in the dams and offspring (Althoff and Grandjean 1979; Althoff et al. 1977a). Weekly intraperitoneal injections of 40 mg N-nitrosodi-n-propylamine produced death due to hepatocellular carcinomas in monkeys after an average duration of 28 months (Adamson and Sieber 1979, 1983).

The U.S. Department of Health and Human Services categorized N-nitrosodi-n-propylamine as reasonably anticipated to be a human carcinogen (NTP 2016), EPA categorized it as a probable human carcinogen (Group B2) (IRIS 2002), and the International Agency for Research on Cancer categorized it as possibly carcinogenic to humans (group 2B) (IARC 1987).

2.20 GENOTOXICITY

Genotoxicity of N-nitrosodi-n-propylamine has been demonstrated consistently in numerous *in vitro* studies summarized in Table 2-2. The *in vitro* assays generally required addition of an exogenous metabolic activation system for expression of effects. As indicated in Table 2-2, N-nitrosodi-n-propylamine was mutagenic in several studies using *Salmonella typhimurium* (Araki et al. 1984; Bartsch et al. 1976, 1980; Dahl 1985; Guttenplan and Hu 1984; Guttenplan 1987; Kirkland et al. 2005; McMahon et al. 1979; Mersch-Sundermann et al. 1994; Moore et al. 1985; Okochi et al. 1997; Phillipson and Ioannides 1985; Probst et al. 1981; Rao et al. 1979, 1982; Yahagi et al. 1977), *Escherichia coli (*Araki et al. 1984; McMahon et al. 1979; Mersch-Sundermann et al. 1994; Nakajima et al. 1974; Rao et al. 1981, 1982), mouse lymphoma cells (Amacher and Paillet 1982, 1983; Amacher et al. 1979), and Chinese hamster V79 cells (Bartsch et al. 1980; Jones and Huberman 1980; Kuroki et al. 1977; Langenbach 1986).

Table 2-2. Genotoxicity of N-Nitrosodi-n-Propylamine *In Vitro*

Species (test system)	Endpoint	Results		Reference
		Activation		
		With	Without	
Prokaryotic organisms				
Salmonella typhimurium	Gene mutation	+	–	Araki et al. 1984
S. typhimurium	Gene mutation	+	–	Bartsch et al. 1976, 1980
S. typhimurium	Gene mutation	+	–	Dahl 1985
S. typhimurium	Gene mutation	+	–	Guttenplan and Hu 1984
S. typhimurium	Gene mutation	+	–	Guttenplan 1987
S. typhimurium	Gene mutation	+	NA	Kirkland et al. 2005
S. typhimurium	Gene mutation	+	–	McMahon et al. 1979
S. typhimurium (strain TA1330)	Gene mutation	+	NA	Mersch-Sundermann et al. 1994
S. typhimurium	Gene mutation	+	–	Moore et al. 1985
S. typhimurium (strain 1535)	Gene mutation	+	NA	Okochi et al. 1997
S. typhimurium	Gene mutation	+	–	Phillipson and Ioannides 1985
S. typhimurium	Gene mutation	+	–	Probst et al. 1981
S. typhimurium	Gene mutation	+	–	Rao et al. 1979
S. typhimurium	Gene mutation	+	–	Rao et al. 1982
S. typhimurium	Gene mutation	+	–	Yahagi et al. 1977
Escherichia coli	Gene mutation	+	–	Araki et al. 1984
E. coli	Gene mutation	+	–	McMahon et al. 1979
E. coli (strain PQ37)	Gene mutation (SOS chromotest)	+	NA	Mersch-Sundermann et al. 1994
E. coli	Gene mutation	+	–	Nakajima et al. 1974
E. coli	Gene mutation	+	–	Rao et al. 1981, 1982
Eukaryotic organisms				
Mouse lymphoma L5178Y cells	Gene mutation	+	–	Amacher et al. 1979
Mouse lymphoma L5178Y cells	Gene mutation	+	–	Amacher and Paillet 1982, 1983
Chinese hamster V9 cells	Gene mutation	+	–	Bartsch et al. 1980
Chinese hamster V9 cells	Gene mutation	+	–	Jones and Huberman 1980
Chinese hamster V9 cells	Gene mutation	+	–	Kuroki et al. 1977
Chinese hamster V9 cells	Gene mutation	+	–	Langenbach 1986
Human hepatocytes	DNA fragmentation	+	NA	Brambilla et al. 1987b
Human hepatocytes	DNA fragmentation	+	NA	Knasmüller et al. 1998
Human hepatocytes	DNA fragmentation	+	NA	Martelli et al. 1988

Table 2-2. Genotoxicity of N-Nitrosodi-n-Propylamine *In Vitro*

		Results		
		Activation		
Species (test system)	Endpoint	With	Without	Reference
Human kidney cells	DNA fragmentation	+	NA	Robbiano et al. 1996
Rat hepatocytes	DNA fragmentation	+	NA	Bradley and Dysart 1981a, 1981b
Rat hepatocytes	DNA fragmentation	+	NA	Bradly et al. 1982
Rat hepatocytes	DNA fragmentation	+	NA	Parodi et al. 1982
Rat hepatocytes	DNA fragmentation	+	NA	Martelli et al. 1988
Rat kidney cells	DNA fragmentation	+	NA	Robbiano et al. 1996
Rat hepatocytes	DNA Repair	+	NA	Yamazaki et al. 1985
Human hepatocytes	Unscheduled DNA synthesis	+	NA	Martelli et al. 1988
Rat hepatocytes	Unscheduled DNA synthesis	+	NA	Martelli et al. 1988
Rat hepatocytes	Unscheduled DNA synthesis	+	NA	Probst et al. 1981
Rat hepatocytes	Unscheduled DNA synthesis	+	NA	Shu and Hollenberg 1996
Mouse HeLa cells	Unscheduled DNA synthesis	+	–	Martin et al. 1978
Chinese hamster fibroblasts	Chromosome aberrations	+	–	Kaneko et el. 1978
Chinese hamster lung cells	Chromosome aberrations	(+)	–	Matsuoka et al. 1979

+ = positive results; (+) = weakly positive results; – = negative results; DNA = deoxyribonucleic acid; NA = not applicable

Deoxyribonucleic acid (DNA) damage (fragmentation) was observed in human hepatocytes and kidney cells (Brambilla et al. 1987b; Knasmüller et al. 1998; Martelli et al. 1988; Robbiano et al. 1996) and in rat hepatocytes and kidney cells (Bradley and Dysart 1981a, 1981b; Bradly et al. 1982; Parodi et al. 1982; Martelli et al. 1988; Robbiano et al. 1996; Yamazaki et al. 1985) in the presence of metabolic activation. Studies evaluating unscheduled DNA synthesis also produced positive results in human hepatocytes (Martelli et al. 1988), rat hepatocytes (Martelli et al. 1988; Probst et al. 1981; Shu and Hollenberg 1996), and mouse HeLa cells (Martin et al. 1978). In the study by Shu and Hollenberg (1996), the extent of DNA damage was significantly increased in hepatocytes of rats pre-treated with phenobarbital and pyridine, demonstrating a correlation between cytochrome P450 activity and genotoxic potency. N-Nitrosodi-n-propylamine also induced chromosome aberrations in Chinese hamster fibroblasts and lung cells (Kaneko et al. 1978; Matsuoka et al. 1979) in the presence of metabolic activation.

As indicated in Table 2-3, N-nitrosodi-n-propylamine produced positive results in several *in vivo* studies in experimental animals. Single doses of 0.31–25 mg/kg N-nitrosodi-n-propylamine produced dose-

related DNA fragmentation in rats treated orally (Brambilla et al. 1981, 1987a), sister chromatid exchanges in mice administered 172 mg/kg via intraperitoneal injection (Parodi et al. 1983), and DNA synthesis suppression in the tubular and renal epithelia of mice treated by intraperitoneal injection (Amlacher and Rudolph 1981). In addition, intraperitoneal injection of 133 mg/kg of N-nitrosodi-n-propylamine to rats resulted in propylation of DNA and ribonucleic acid (RNA), an event regarded as critical in the initiation of carcinogenesis by this and related alkylating agents (Park et al. 1980). In a repeated exposure study, gavage administration of N-nitrosodi-n-propylamine for 14 days resulted in increases the number of micronucleated hepatocytes in rats, but did not alter the number of micronucleated immature erythrocytes (Terashima et al. 2015).

Table 2-3. Genotoxicity of N-Nitrosodi-n-Propylamine *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Drosophila melanogaster	Host-mediated DNA-repair assay	+	Knasmüller et al. 1990
Rat (liver)	DNA alkylation	+	Park et al. 1980
Rat (hepatocytes)	DNA fragmentation	+	Brambilla et al. 1981, 1987a
Mouse (liver and renal epithelial cells)	Suppressed DNA synthesis	+	Amlacher and Rudolph 1981
Mouse (bone marrow)	Sister chromatid exchange	+	Parodi et al. 1983
Rat (hepatocytes)	Micronucleus assay	–	Hamada et al. 2015
Rat (bone marrow/peripheral blood)	Micronucleus assay	–	Hamada et al. 2015
Rat (hepatocytes)	Micronucleus assay	+	Terashima et al. 2015
Rat (bone marrow)	Micronucleus assay	–	Terashima et al. 2015
Mouse (bone marrow)	Micronucleus assay	–	Morita et al. 1997
Mouse (peripheral blood)	Micronucleus assay	–	Suzuki et al. 1999

+ = positive results;– = negative results; DNA = deoxyribonucleic acid

In contrast to much of the data, micronucleus assays in rats and mice produced mixed results. Hamada et al. (2015) reported positive results for induction of micronuclei in hepatocytes, but negative results in bone marrow of rats administered 10–40 mg/kg N-nitrosodi-n-propylamine via gavage for 14 days. Similarly, Morita et al. (1997) reported negative results for induction of micronuclei in bone marrow of mice interperitoneally injected with 50–400 mg/kg N-nitrosodi-n-propylamine. Negative results were also reported for induction of micronuclei in peripheral blood of mice interperitoneally injected with 250 mg/kg, although increases in *LacZ* mutation frequency in the liver, lung, and kidney (target organs for carcinogenesis) were observed (Suzuki et al. 1999).

A single host-mediated DNA repair assay was available (Knasmüller et al. 1990). *Drosophila Melanogaster* were simultaneously injected with a mixture of two *E. coli* strains (*uvrB/recA* and *uvr+/rec+*) and 0.5–10.5 mmol/L solutions of N-nitrosodi-n-propylamine. DNA damage was reported within 3 hours after injection and there was a dose-dependent differential killing effect.