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 N-nitrosodi-n-propylamine. Its purpose is to present levels of significant exposure for N-nitrosodi-n-propylamine 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 N-nitrosodi-n-propylamine and (2) a depiction of significant exposure levels associated with various adverse health effects.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF FXPOSURE

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.

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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

No studies were located regarding the following effects in humans or animals following inhalation exposure to N-nitrosodi-n-propylamine:

2.2.1.1 Death

2.2.1.2 Systemic Effects

2.2.1.3 Neurological Effects

2.2.1.4 Immunological Effects

2.2.1.5 Developmental Effects

2.2.1.6 Reproductive Effects

2.2.1.7 Genotoxic Effects

2.2.1.8 Cancer

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding lethality in humans following oral exposure to N-nitrosodi-n-propylamine.

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. The 480 mg/kg LD_{50} is indicated in

Table 2-1 and Figure 2-l. No short-term studies of N-nitrosodi-n-propylamine administered in drinking water were located; therefore, the dose level of 480 mg/kg, which was administered by gavage in water (Druckrey et al. 1967), was converted to an equivalent concentration of 3400 ppm in water for presentation in Table l-4.

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 2.2.2.8, Oral exposure, Cancer). The 5.1, 6.3 and 12.6 mg/kg/day doses are serious LOAEL values for lethality in rats due to intermediate duration oral exposure and are recorded in Table 2-l and plotted in Figure 2-l. No studies were located regarding survival in animals following chronic oral exposure to N-nitrosodi-n-propylamine.

2.2.2.2 Systemic Effects

No studies were located regarding systemic effects in humans following oral exposure to N-nitrosodi-n-propylamine.

Hepatic Effects. 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-l and Figure 2-l as a serious LOAEL for hepatic effects in rats due to acute oral exposure. No short-term studies of N-nitrosodi-n-propylamine administered in drinking water were located; therefore, the dose level of 480 mg/kg, which was administered by gavage in water (Druckrey et al. 1967), was converted to an equivalent concentration of 3400 ppm in water for presentation in Table l-4.

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 four-day study. N-nitrosodi-n-propylamine treatment resulted in significantly prolonged PST in both studies. Liver histology was evaluated in the four-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-

TABLE 2-1. Levels of Significant Exposure to N-Nitrosodi-n-propylamine - Oral

Graph Key	Species[a]	Route	Duration/ Frequency Exposure	Effect	NOAEL[b] (mg/kg/day)	LOAEL[c] (Effect)		Reference
						Less Serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE								
Death								
1	rat	(G)	one dose				480 (LD_{50})	Druckrey et al. 1967
Systemic								
2	mouse	(W)	1 wk, daily	Hepatic	9.5[d]			Tyndall et al. 1978
3	mouse	(G)	4 d, once/day	Hepatic		40 (increased PST)		Nishie et al. 1972
4	rat	(G)	one dose	Hepatic			480 (necrosis)	Druckrey et al. 1967
INTERMEDIATE EXPOSURE								
Death								
5	rat	(W)	30 wk, 5 d/wk				5.1 (decreased longevity)	Lijinsky and Taylor 1978, 1979
6	rat (male)	(G)	30 wk, 2 d/wk				12.6 (decreased longevity)	Lijinsky and Reuber 1983
7	rat (female)	(G)	30 wk, 2 d/wk				6.3 (decreased longevity)	Lijinsky and Reuber 1983
Cancer								
8	rat	(W)	30 wk, 5 d/wk				2.6 (CEL[e]-esophagus, forestomach tumors)	Lijinsky and Reuber 1981
9	rat	(G)	30 wk, 2 d/wk				6.3 (CEL[e]-liver, nasal, esophagus tumors)	Lijinsky and Reuber 1983

TABLE 2-1 (continued)

Graph Key	Species[a]	Route	Duration/ Frequency Exposure	Effect	NOAEL[b] (mg/kg/day)	LOAEL[c] (Effect) Less Serious (mg/kg/day)	LOAEL[c] (Effect) Serious (mg/kg/day)	Reference
10	rat	(F)	life, daily				4 (CEL[e]-liver carcinoma)	Druckrey et al. 1967
11	mouse	(G)	50 wk, 2 d/wk				1 (CEL[e]-forestomach, pulmonary tumors)	Griciute et al. 1982

[a]G - gavage, W - water, F - feed
[b]NOAEL - No Observed Adverse Effect Level
[c]LOAEL - Lowest Observed Adverse Effect Level
[d]Used to derive acute oral MRL; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability), resulting in a MRL of 0.095 mg/kg/day. This MRL has been converted to an equivalent concentration in water (3.3 ppm) for presentation in Table 1-3.
[e]CEL - Cancer Effect Level

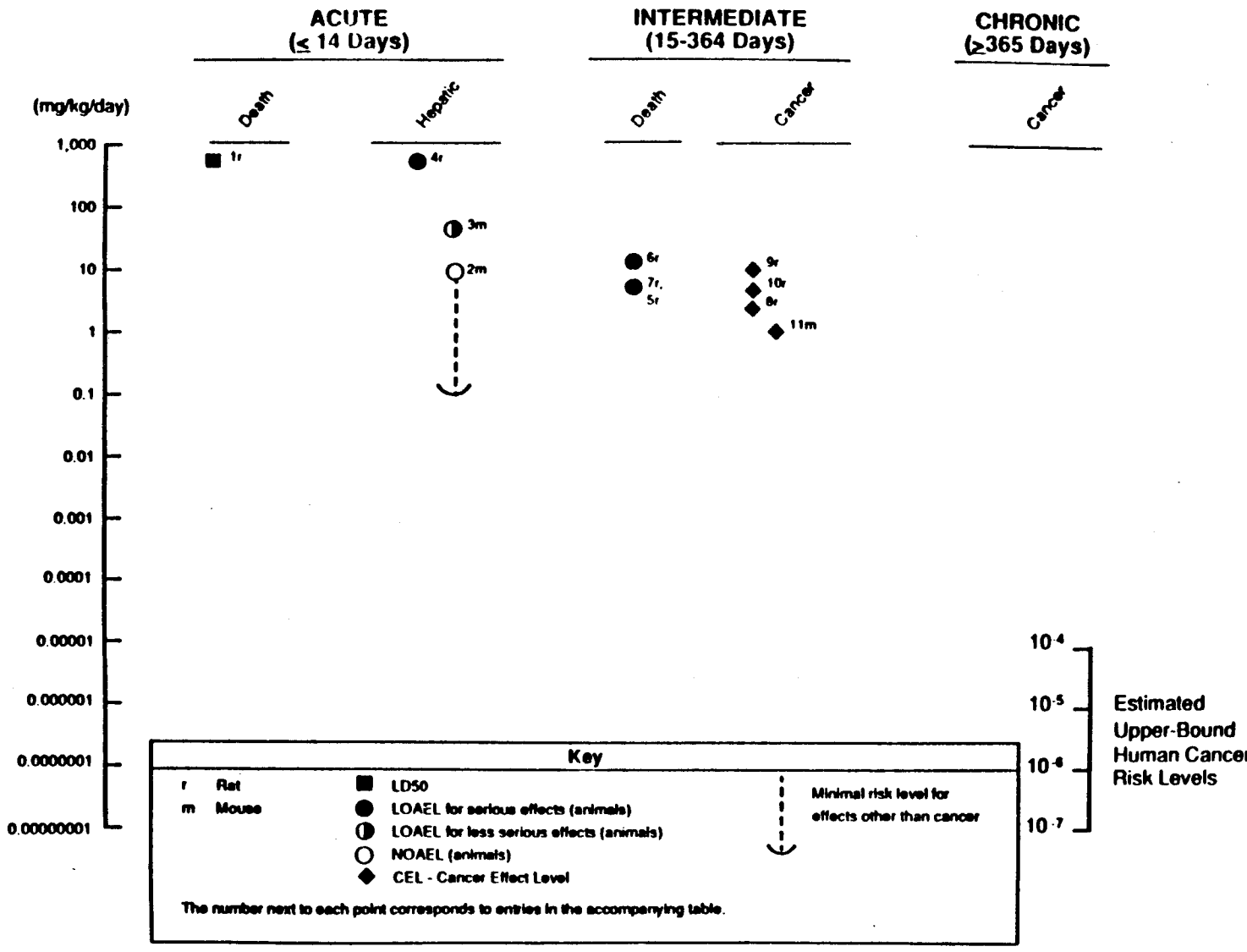

FIGURE 2-1. Levels of Significant Exposure to N-Nitrosodi-n-propylamine - Oral

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 four-day study, 40 mg/kg/day can be regarded as a LOAEL for less serious hepatic effects due to acute oral exposure (Table 2-l and Figure 2-l). No short-term studies of N-nitrosodi-n-propylamine administered in food were located; therefore, the dose level of 40 mg/kg/day, which was administered by gavage in olive oil (Nishie et al. 1972), was converted to an equivalent concentration of 308 ppm in food for presentation in Table l-4.

Liver histology and activities of liver-associated serum enzymes (SGOT, alkaline phosphatase, lactate dehydrogenase, gamma-glutamyl-transferase) were unaltered in mice exposed to 9.5 mg/kg/day via drinking water for one week (Tyndall et al. 1978). This dose represents a NOAEL for hepatic effects due to acute duration exposure (Table 2-l and Figure 2-l). Because this NOAEL is lower than the 40 mg/kg/day LOAEL for hepatic effects (Nishie et al. 1972), it can be used as the basis for an acute MRL (Figure 2-l). Based on this value, an acute oral MRL of 0.095 mg/kg/day was calculated, as described in the footnote in Table 2-l. This MRL has been converted to an equivalent concentration in drinking water (3.3 ppm) for presentation in Table 1-3.

Other Effects. Plasma esterase profiles were examined in mice exposed to various carcinogenic, weakly carcinogenic and noncarcinogenic chemicals in the drinking water for one week (Tyndall et al. 1978). N-nitrosodi-n-propylamine, administered at a dose 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.

No studies were located regarding the following effects in humans or animals following oral exposure to N-nitrosodi-n-propylamine:

2.2.2.3 Immunological Effects

2.2.2.4 Neurological Effects

2.2.2.5 Developmental Effects

2.2.2.6 Reproductive Effects

2.2.2.7 Genotoxic Effects

Single doses of N-nitrosodi-n-propylamine, administered by gavage, produced fragmentation of liver DNA in rats (Brambilla et al. 1981, 1987a). Doses ranged from 0.31 to 25 mg/kg and the effect was dose-related.

2.2.2.8 Cancer

No studies were located regarding carcinogenic effects in humans following oral exposure to N-nitrosodi-n-propylamine.

The carcinogenicity of N-nitrosodi-n-propylamine has been demonstrated unequivocally 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-npropylamine 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 30-40%. 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 2.2.2.1 (Oral exposure, Death), tumor development in all of the rat studies was life-shortening.

The lowest drinking water and gavage doses of N-nitrosodi-n-propylamine that were carcinogenic to rats are 2.6 mg/kg/day (Lijinsky and Reuber 1981) and 6.3 mg/kg/day (Lijinsky and Reuber 1983), respectively; these are intermediate duration effect levels for carcinogenicity (cancer effects levels, CELs) because exposure durations were 30 weeks (Table 2-l and Figure 2-l). The lowest dose tested in the study of Druckrey et al. (1967) (4 mg/kg/day) is also presented in Table 2-l and Figure 2-l in the intermediate duration category as this study is used as the basis for a carcinogenic potency factor for N-nitrosodi-n-propylamine (EPA 1988). The 4 mg/kg/day dose from the Druckrey et al. (1967) study is considered to be the lowest CEL due to intermediate duration exposure because (1) time to tumor data suggest that survival was generally less than one year, and (2) survival was less than one year in the other cancer studies which used

similar or lower doses. Although a carcinogenic potency factor is based on this study, it should be recognized that the study is limited by small numbers of treated rats, no controls and unreported specific tumor incidences. Using hepatocellular carcinoma response data from this study, EPA (1988) derived and verified an oral slope factor (BH) of 7.0 $(mg/kg/day)^{-1}$ for N-nitrosodi-n-propylamine. Using this slope factor the doses associated with upper bound lifetime cancer risk levels of 10^{-4} to 10^{-7} are calculated to be 1.4×10^{-5} to 1.4×10^{-8} mg/kg/day, respectively. The cancer risk levels are plotted in Figure 2-l in the chronic duration category because they represent lifetime risks for humans.

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. The 1 mg/kg/day dose from the Griciute et al. (1982) study represents an intermediate duration CEL in mice (Table 2-l and Figure 2-l).

2.2.3 Dermal Exposure

No studies were located regarding the following effects in humans or animals following dermal exposure to N-nitrosodi-n-propylamine:

2.2.3.1 Death

2.2.3.2 Systemic Effects

2.2.3.3 Neurological Effects

2.2.3.4 Immunological Effects

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

2.2.3.8 Cancer

2.3 RELEVANCE TO PUBLIC HEALTH

Death. Information regarding death of humans following exposure to N-nitrosodi-n-propylamine by any route was not found. Case reports indicate that intentional oral and accidental inhalation exposures to unknown levels of N-nitrosodimethylamine, however, have resulted in deaths in humans (Barnes and Magee 1954, Cooper and Kimbrough 1980, Freund 1937, Fussgaenger and Ditschuneit 1980, Pedal et al. 1982); these deaths apparently were due

to hepatotoxicity. Limited data are available for acute lethality in N-nitrosodi-n-propylamine-exposed animals. In the only acute study that used a natural route of exposure, an oral LD_{50} value of 480 mg/kg was determined for rats (Druckrey et al. 1967). Subcutaneous injection LD_{50} values have been determined for N-nitrosodi-n-propylamine in various species; these are consistent with the oral LD_{50} and include 487.2 mg/kg for rats (Reznik et al. 1975), 689 mg/kg for mice (Dickhaus et al. 1977) and 600 mg/kg for hamsters (Pour et al. 1973). Pathologic examinations revealed centrilobular liver necrosis and hemorrhages in the lungs, stomach, kidneys and/or heart.

Oral administration of N-nitrosodi-n-propylamine at doses ranging from 5.1-12.6 mg/kg/day, on 2 or 5 days a week for 30 weeks, produced high mortality in rats (Lijinsky and Reuber 1983; Lijinsky and Taylor 1978, 1979). Once-weekly subcutaneous injections of similar doses of N-nitrosodin-propylamine to rats ($\leq$24.4 mg/kg) (Reznik et al. 1975), mice ($\geq$34.5 mg/kg) (Dickhaus et al. 1977) and hamsters ($\geq$3.75 mg/kg) (Pour et al. 1973, Althoff et al. 1973a,b) also were life-shortening (average survival times of 27-54 weeks) . Weekly intraperitoneal injection of 40 mg/kg N-nitrosodi-n-propylamine produced deaths in monkeys after an average period of 28 months (Adamson and Sieber 1979, 1983). Mortality in the above studies is dose-related and due to tumor development.

The available lethality data indicate that deaths resulting from acute exposure to N-nitrosodi-n-propylamine are due primarily to hepatotoxicity, that deaths resulting from repeated exposure to N-nitrosodi-n-propylamine are due to tumors occurring primarily in the liver, and that causes of death and lethal doses are similar in different species. The causes of death produced by N-nitrosodi-n-propylamine also are consistent with those produced by other dialkylnitrosamines (Magee et al. 1976).

Systemic Effects. Information regarding systemic effects in humans following exposure to N-nitrosodi-n-propylamine was not found. Very limited information is available for systemic effects of N-nitrosodi-n-propylamine in animals because interest in this compound has focused overwhelmingly on carcinogenicity.

As indicated in the previous subsection (see Death above), lethal single oral or subcutaneous doses of N-nitrosodi-n-propylamine produced hepatic necrosis and hemorrhagic lesions in the liver and other internal tissues in rats and hamsters (Druckrey et al. 1967, Pour et al. 1973, Reznik et al. 1975). Similar effects were reported by Nishie et al. (1972), who observed that gavage doses of 40 mg/kg/day for 4 consecutive days produced swelling of hepatocytes and possibly necrosis in the centrilobular area of the liver in mice. Hepatotoxicity and hemorrhagic lesions in the liver and other internal tissues are also the primary acute effects of other dialkylnitrosamine compounds (Magee et al. 1976). Based on data for other dialkylnitrosamines, it can be inferred that systemic effects of intermediate or chronic duration exposure to N-nitrosodi-n-propylamine are likely to include acute-type responses and preneoplastic alterations.

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Although it is apparent that N-nitrosodi-n-propylamine produces hepatotoxicity and hemorrhages in the lungs, stomach, kidney and heart at acute lethal doses in animals, there is only limited information regarding the threshold for these effects following acute exposure and documentation of these effects following intermediate or chronic duration exposure is not available. Although human data are not available, human fatalities due to intentional oral and accidental inhalation exposures to unknown levels of N-nitrosodimethylamine have been described in case reports in which hemorrhagic, necrotic and cirrhotic alterations in the liver and diffuse internal bleeding were observed (Barnes and Magee 1954; Cooper and Kimbrough 1980; Freund 1937; Fussgaenger and Ditschuneits 1980; Pedal et al. 1982). The available information for N-nitrosodi-n-propylamine and related nitrosamines therefore indicates that N-nitrosodi-n-propylamine is likely to produce characteristic hepatic and/or hemorrhagic effects in humans exposed orally or by inhalation. Systemic effects of N-nitrosodi-n-propylamine may result from dermal exposure, since evidence indicates that dermal absorption of N-nitrosodi-n-propylamine is likely (Section 2.6.1.3, Absorption, Dermal exposure).

Developmental Effects. Limited information regarding developmental effects of N-nitrosodi-n-propylamine in humans or in animals is available from subcutaneous injection transplacental carcinogenesis studies with hamsters (Althoff et al. 1977a; Althoff and Grandjean 1979). 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 four 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. No studies were located demonstrating that N-nitrosodi-n-propylamine crosses the placenta in humans and it is not known whether N-nitrosodi-n-propylamine can cause developmental effects in humans. It is relevant to note, however, that limited evidence indicates that N-nitrosodimethylamine is fetotoxic but not teratogenic. Also, it has been estimated from studies with rodents that drugs with a molecular weight of less than 1,000 can readily cross the placenta (Mirkin 1973); the molecular weight of N-nitrosodi-n-propylamine is 130.2.

Genotoxic Effects. No studies were located regarding the genotoxicity of N-nitrosodi-n-propylamine in humans by the inhalation, oral or dermal routes. Fragmentation of DNA was observed, however, in human hepatocytes cultured in the presence of N-nitrosodi-n-propylamine (Brambilla et al. 1987b).

Genotoxicity of N-nitrosodi-n-propylamine has been demonstrated consistently in numerous in vitro studies. As indicated in Table 2-2,

TABLE 2-2. Genotoxicity of N-Nitrosodi-n-Propylamine In Vitro

Endpoint	Species (Test System)	Result: With Activation	Result: Without Activation	References
Gene mutation	Salmonella typhimurium	+	-	Yahagi et al. 1977, Bartsch et al. 1976, 1980, McMahon et al. 1979, Rao et al. 1979, Araki et al. 1984, Phillipson and Ioannides 1985, Guttenplan and Hu 1984, Guttenplan 1987, Moore et al. 1985, Dahl 1985, Rao et al. 1982, Probst et al. 1981
	Escherichia coli	+	-	McMahon et al. 1979, Araki et al. 1984, Nakajima et al. 1974, Rao et al. 1981, 1982
	Mouse lymphoma L5178Y cells	+	-	Amacher et al. 1979, Amacher and Paillet 1982, 1983,
	Chinese hamster V79 cells	+	-	Kuroki et al. 1977, Bartsch et al. 1980, Jones and Huberman 1980, Langenbach 1986
DNA fragmentation	Rat hepatocytes	+	NT	Bradley and Dysart 1981a,b, Bradley et al. 1982, Parodi et al. 1982
	Human hepatocytes	+	NR	Brambilla et al. 1987b
Unscheduled DNA synthesis	Rat hepatocytes	+	NT	Probst et al. 1981
	HeLa cells	+	-	Martin et al. 1978
DNA repair	Rat hepatocytes	+	NT	Yamazaki et al. 1985
Chromosome aberrations	Chinese hamster fibroblasts	+	-	Kaneko et al. 1978
	Chinese hamster lung cells	(+)	-	Matsuoka et al. 1979

NT = not tested; NR = not reported

N-nitrosodi-n-propylamine was mutagenic in bacteria (Salmonella Typhimurium, Escherichia coli) and mammalian cells (mouse lymphoma L5178Y, Chinese hamster V79), caused DNA effects (fragmentation, unscheduled synthesis, repair) in rat hepatocytes, and chromosome aberrations in Chinese hamster cells. The in vitro assays generally required addition of an exogenous metabolic activation system for expression of effects; this is consistent with the apparent indirect carcinogenicity of N-nitrosodi-npropylamine. Single doses of N-nitrosodi-n-propylamine produced DNA fragmentation in rats treated orally and sister chromatid exchange and DNA synthesis suppression in mice treated by intraperitoneal injection (Table 2-3). In addition, intraperitoneal injection (133 mg/kg) of N-nitrosodi-npropylamine to rats results in propylation of DNA and RNA, an event regarded as critical in the initiation of carcinogenesis by this and related alkylating agents (Park et al. 1980).

The weight of evidence indicates that N-nitrosodi-n-propylamine is genotoxic in mammalian cells. The effect observed in the study with human hepatocytes (DNA fragmentation) (Brambilla et al. 1987b) is consistent with the results of other assays, particularly the in vitro and in vivo rat hepatocyte DNA fragmentation assays (Table 2-2 and 2-3). Given the type and weight of genotoxicity evidence, one can predict that N-nitrosodi-n-propylamine poses a genotoxic threat to humans.

Cancer. Information regarding the carcinogenicity of N-nitrosodi-npropylamine in humans was not located. In animals, carcinogenicity of N-nitrosodi-n-propylamine has been demonstrated in several species in all studies that have been conducted. In rats observed for life, daily or partial weekly (2 days/week or 5 days/week) oral exposure produced tumors primarily in the liver, nasal cavity and esophagus (Druckrey et al. 1967; Lijinsky and Reuber 1981, 1983; Lijinsky and Taylor 1978, 1979). In mice, increased incidences of forestomach tumors occurred as a result of twice weekly orally treatment for 50 weeks (Griciute et al. 1982). 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 et al. 1977a; Althoff and Grandjean 1979). Weekly intraperitoneal injections of 40 mg N-nitrosodin-propylamine produced death due to hepatocellular carcinomas in monkeys after an average duration of 28 months (Adamson and Sieber 1979, 1983).

Overall, there is conclusive evidence that N-nitrosodi-n-propylamine is carcinogenic in animals. The carcinogenicity of N-nitrosodi-n-propylamine may be related to alkylation of DNA. It is important to recognize that cancer was observed in the oral and injection studies after durations as short as 20-30 weeks, and that once weekly oral exposures and single

TABLE 2-3. Genotoxicity of N-Nitrosodi-n-propylamine In Vivo

Endpoint	Species (Test System)	Result	References
DNA alkylation	Rat liver	+	Park et al. 1980
DNA fragmentation	Rat hepatocytes	+	Brambilla et al. 1981, 1987a
Suppressed DNA synthesis	Mouse liver and renal epithelial cells	+	Amlacher and Rudolph 1981
Sister chromatid exchange	Mouse bone marrow cells	+	Parodi et al. 1983

exposure by injection were sufficient to induce cancer. Based on the evidence of carcinogenicity in animals, it is reasonable to anticipate that N-nitrosodi-n-propylamine will be carcinogenic in humans.

2.4 LEVELS IN HUMAN TISSUES AND FLUIDS ASSOCIATED WITH HEALTH EFFECTS

There is no known association between levels of N-nitrosodi-npropylamine or its metabolites in human tissues and fluids and health effects of N-nitrosodi-n-propylamine. N-nitrosodi-n-propylamine was detected in the liver of 1 of 4 deceased subjects (Cooper et al. 1987). As indicated in Section 2.6.2 (Distribution), the cause of death is not attributable to N-nitrosodi-n-propylamine.

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

There is no known association between levels of N-nitrosodi-npropylamine in the environment and levels of N-nitrosodi-n-propylamine or its metabolites in human tissues and fluids or health effects of N-nitrosodi-n-propylamine.

2.6 TOXICOKINETICS

2.6.1 Absorption

2.6.1.1 Inhalation Exposure

No studies were located regarding absorption in humans or animals following inhalation exposure to N-nitrosodi-n-propylamine. However, structurally similar compounds, such as, N-nitrosodimethylamine and N-nitrosodiethanolamine, are readily absorbed (70-90% of the dose) following inhalation exposure in experimental animals (Klein and Schmezer 1984; Preussmann et al. 1981). Absorption was inferred by monitoring urinary excretion of the unchanged compounds.

2.6.1.2 Oral Exposure

No studies were located regarding absorption in humans following oral exposure to N-nitrosodi-n-propylamine.

Specific information regarding absorption in animals following oral exposure to N-nitrosodi-n-propylamine was not located. Gastrointestinal absorption of N-nitrosodi-n-propylamine by rodents is indicated by the occurrence of metabolites in the urine following oral treatment (Section 2.3.1.3) and systemic effects in oral carcinogenicity and toxicity studies (Section 2.2). Other nitrosamines are rapidly absorbed from the gastrointestinal tract after oral exposure. Diaz Gomez et al. (1977) found that less than 2% of radiolabelled dimethylnitrosamine could be recovered

from the stomach and intestine of rats 15 minutes after administration. Also in rats, Lijinsky et al. (1981) and Preussmann et al. (1978) estimated absorption rates of 25% and 70% of the dose for N-nitrosodiethanolamine, respectively (estimates are from urinary excretion).

2.6.1.3 Dermal Exposure

No studies were located regarding absorption in humans following dermal exposure to N-nitrosodi-n-propylamine. However, absorption of N-nitroso-di-n-propylamine through human skin in vivo (Edwards et al. 1979) and in vitro (Bronaugh et al. 1979, 1981) has been demonstrated.

Diffusion of N-nitroso-di-n-propylamine through rat skin in vitro has been demonstrated (Wishnok et al. 1982). Information regarding dermal absorption of N-nitroso-di-n-propylamine by animals in vivo was not located in the reviewed literature. Dermal absorption of N-nitrosodiethanolamine has been determined in pigs (Marzulli et al. 1981), monkeys (Marzulli et al. 1981), and rats (Airoldi et al. 1984; Lijinsky et al. 1981). The degree of absorption varied greatly (4-78%) depending on the site of the application and the vehicle used. Based on the data for N-nitrosodi-n-propylamine and N-nitrosodiethanolamine, it is likely that N-nitrosodi-n-propylamine will be absorbed following dermal exposure.

2.6.2 Distribution

Route-specific distribution data for N-nitrosodi-n-propylamine in humans were not located in the reviewed literature. Quantitative analyses of six volatile nitrosamines in postmortem organs (brain, liver, kidneys, pancreas) from four human subjects were conducted (Cooper et al. 1987). N-nitrosodi-n-propylamine was detected only in the liver of one of the subjects (female, age 84 years) at a concentration of 19.30 ng/50 g of tissue. The ages of the other subjects (two males, one female) ranged from 47-80 years. Unusual sources of nitrosamine exposure or causes of death were not indicated.

Transplacental transport of N-nitrosodi-n-propylamine was shown in pregnant hamsters (Althoff et al. 1977a, Althoff and Grandjean 1979). After a single 100 mg/kg subcutaneous injection, N-nitrosodi-n-propylamine was detected in the maternal blood, placenta, fetus, and amniotic fluid. The concentration of the chemical in maternal blood reached a maximum at 45 and 90 minutes after the injection, whereas a single peak at 90 minutes was observed in the fetus. Analysis for metabolites was not conducted, but 1.6% of the unchanged compound was found in the placenta and 1.3% in the fetus at day 14 of gestation. Detection of 0^6-methylguanine in human placental DNA by immunoassay indicates that nitrosamines, as a group, can reach the placenta in humans (Foiles et al. 1988).

Limited data are available regarding the distribution of related nitrosamines. Daugherty and Klapp (1976) reported that after oral administration of ^{14}C-N-nitrosodimethylamine to mice radioactivity could be

detected in the homogenates of heart, forestomach, esophagus, liver and lungs. Radioactivity was detected in all organs and tissues of rats after oral doses of ^{14}C-N-nitrosodiethanolamine (Lethco et al. 1982). After intravenous injection of ^{14}C-N-nitrosodi-n-butylamine to rats the highest concentrations of radiolabel occurred in the nasal mucosa, liver and preputial gland (Brittebo and Tjalve 1982).

2.6.3 Metabolism

No studies were located regarding metabolism in humans following exposure to N-nitrosodi-n-propylamine. .

In vitro and in vivo studies with rodents have been conducted that provide evidence that N-nitrosodi-n-propylamine can be metabolized via oxidation at the alpha, beta and gamma carbon positions (Figure 2-2). Alpha carbon oxidation (hydroxylation) is regarded as the primary pathway, resulting in formation of propionaldehyde and 1-propanol and 2-propanol as metabolites (Farrelly et al. 1984; Park and Archer 1978; Park et al. 1977). 1-Propanol and 2-propanol are formed via propyldiazohydroxide and a propyl cation (carbonium ion). It is generally believed that the carbonium ions can also react with nucleic acids to form propylated adducts, but Park et al. (1980) have suggested that propylation takes place via a bimolecular reaction. However, reaction of DNA with propylnitrosourea (a direct acting equivalent of N-nitrosodi-n-propylamine) results in formation of n-propyl and isopropyl DNA adducts, suggesting carbonium ions are involved. Alkylation of nucleic acids and proteins by metabolites of nitrosamines has been suggested as the mechanism responsible for the toxic and carcinogenic properties of these substances.

Beta-carbon hydroxylation yields N-nitroso-2-hydroxy-propylpropylamine which is excreted as the glucuronide or further oxidized to a small extent to N-nitroso-2-oxopropylpropylamine (Bauman et al. 1985; Leung and Archer 1981; Park and Archer 1978; Suzuki and Okada 1981). Methylated hepatic nucleic acids have been recovered from rats and hamsters treated with N-nitrosodi-n-propylamine (Althoff et al. 1977b; Kruger 1971, 1973; Kruger and Bertram 1973; Leung and Archer 1984). Putative methylating intermediates, formed from N-nitroso-2-oxo-n-propylamine, are N-nitrosomethylpropylamine and diazomethane.

Gamma-carbon hydroxylation yields N-nitroso-3-hydroxy-propylpropylamine and its oxidation product, N-propyl-N-(2-carboxyethyl)nitrosamine (Baumann et al. 1985; Blattmann and Preussman 1973, Suzuki and Okada 1981). Urinary N-propyl-N-(2-carboxyethyl)nitrosamine amounted to approximately 5% of a 300 mg/kg oral dose of N-nitrosodi-n-propylamine in rats (Suzuki and Okada 1981).

Documented and postulated metabolites of N-nitrosodi-n-propylamine have been shown to be carcinogenic in hamsters and rats (IARC 1978). These include N-nitroso-bis-(2-hydroxy-n-propyl)amine, N-nitroso-2-oxo-n-

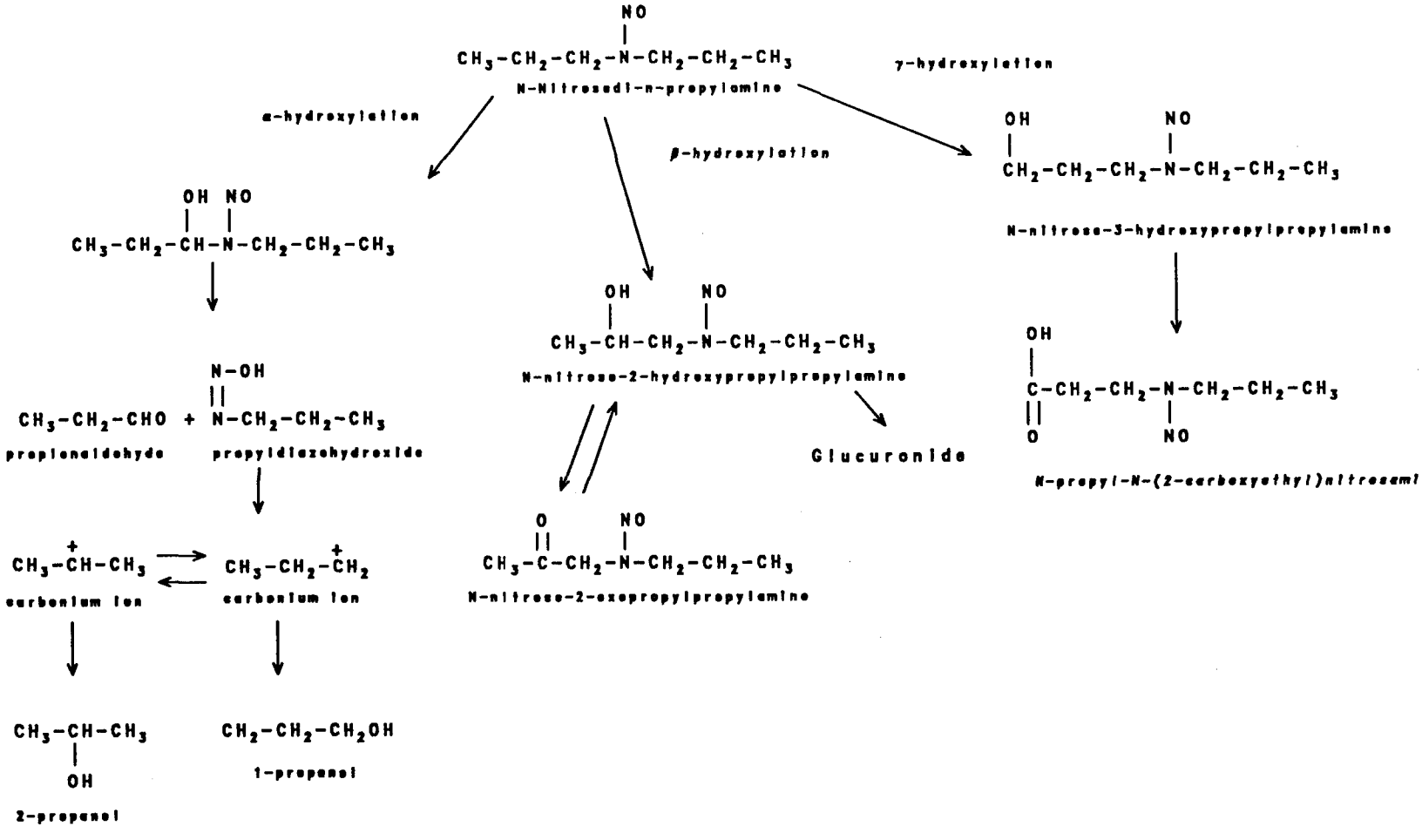

FIGURE 2-2. Metabolism of N-Nitrosodi-n-propylamine

propylpropylamine, N-nitroso-bis(2-oxo-n-propyl)amine and N-nitroso-bis(2-acetoxy-n-propyl)amine. Main tumor sites of many of these metabolites include those associated with N-nitrosodi-n-propylamine treatment (Section 2.2.2.8, Oral exposure, Cancer).

2.6.4 Excretion

2.6.4.1 Inhalation Exposure

No studies were located regarding excretion in humans or animals following inhalation exposure to N-nitrosodi-n-propylamine.

2.6.4.2 Oral Exposure

No studies were located regarding excretion in humans following oral exposure to N-nitrosodi-n-propylamine.

Rats excreted metabolites but not unchanged N-nitrosodi-n-propylamine in the urine following oral dosing with N-nitrosodi-n-propylamine (Blattmann and Preussmann 1973, Suzuki and Okada 1981). The principal metabolite in the Suzuki and Okada (1981) study, N-propyl-N-(2-carboxyethyl)nitrosamine, amounted to approximately 5% of the administered dose. Additional information regarding the extent or rate of excretion in either of the studies was not reported.

2.6.4.3 Dermal Exposure

No studies were located regarding excretion in humans following dermal exposure to N-nitrosodi-n-propylamine.

Excretion of unchanged N-nitrosodiethanolamine in the urine of rats has been reported in several studies after cutaneous application of N-nitrosodiethylamine (Airoldi et al. 1984; Lijinsky et al. 1981; Preussmann et al. 1981).

2.7 INTERACTIONS WITH OTHER CHEMICALS

Ethanol was found to enhance the carcinogenicity of N-nitrosodi-npropylamine. Mice that were treated with estimated 1 mg/kg doses of N-nitrosodi-n-propylamine dissolved in 40% ethanol by gavage, twice a week for 50 weeks, developed higher incidences of tumors than mice that were similarly treated with the same dose of compound given in water (Griciute et al. 1982). The most pronounced tumor enhancement was in the forestomach (51% carcinomas vs. 10% in N-nitrosodi-n-propylamine/water group), but increases in pulmonary adenomas and lymphomas also occurred.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

No populations with unusual susceptibility to health effects of N-nitrosodi-n-propylamine have been identified. However, heavy consumers of alcoholic beverages might be considered to be a susceptible population based on a single report in which ethanol was shown to potentiate the carcinogenicity of N-nitrosodi-n-propylamine in mice (Griciute et al. 1982).

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 N-nitrosodi-n-propylamine 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-Nitrosodi-n-propylamine

Information regarding health effects of N-nitrosodi-n-propylamine in humans is not available. Health effects of N-nitrosodi-n-propylamine in animals have been investigated only in oral exposure studies. As indicated in Figure 2-3, animal oral data are available for lethality, acute systemic effects, genotoxicity and cancer. These data indicate that the acute toxicity of N-nitrosodi-n-propylamine is attributable to hepatic effects and that intermediate duration exposure is life-shortening due to cancer.

2.9.2 Data Needs

Single Dose Exposure. Information on lethality (LD_{50}) and severe systemic effects in rats are available from one single dose oral study. Similar information is available from single dose subcutaneous injection studies with rats, mice and hamsters. Another oral study reported a nonlethal effect (increased pentobarbital sleeping time) but none of the other studies reported non-lethal doses or attempted to identify dose-response data for other subtle systemic effects. Additional single dose oral studies would provide better information on thresholds for lethality and systemic toxicity as well as interspecies differences. Inhalation and dermal studies with N-nitrosodi-n-propylamine have not been conducted; single dose studies involving exposure by these routes would provide information on lethality, systemic effects and skin and eye irritation.

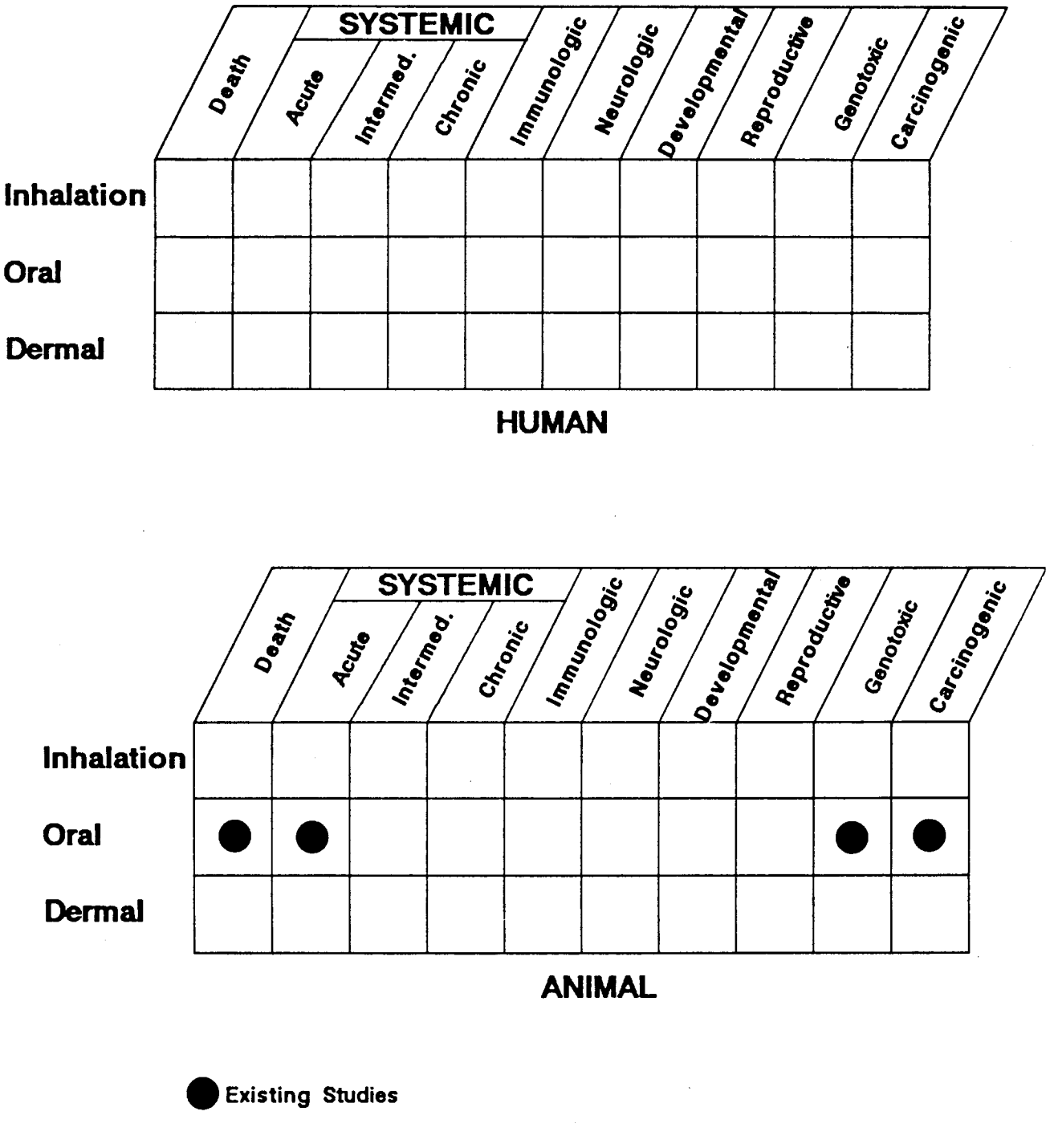

FIGURE 2-3. Existing Information on Health Effects of N-Nitrosodi-n-propylamine

Repeated Dose Exposure. Oral studies provide limited information on the threshold for hepatotoxicity in mice. Several intermediate duration oral studies with rats, one limited oral study with mice and injection studies with rats, mice, hamsters and monkeys provide survival data but noinformation on effects other than cancer. Additional short-term repeated dose oral studies (e.g., 14-28 day studies) in various species could provide additional information on systemic effects, particularly dose-response characterization of hepatic/hemorrhagic effects. Repeated dose inhalation studies are lacking and could provide useful information regarding lethality and systemic effects.

Chronic Exposure and Carcinogenicity. Chronic oral toxicity data for N-nitrosodi-n-propylamine are not available because treated animals have died of cancer within one year of treatment. Animals treated with doses lower than those used in the intermediate duration studies may survive chronic exposure and provide information on nonneoplastic effects. With the exception of a single study with mice, species other than rat have not been tested for carcinogenicity by the oral route.

Genotoxicity. The genotoxicity of N-nitrosodi-n-propylamine is documented but only one in vivo study used an environmentally relevant route of exposure (oral), and only two studies (in vitro) evaluated human cells. Additional studies, particularly types providing information on the potential for heritable mutations in humans, would add to the data base on genotoxicity.

Reproductive Toxicity. Information on the reproductive toxicity of N-nitrosodi-n-propylamine is not available. 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 N-nitrosodi-n-propylamine in animals, which may be related to possible reproductive effects in humans.

Developmental Toxicity. Some data on developmental toxicity are available from single-dose subcutaneous injection studies with hamsters. Developmental effects of N-nitrosodi-n-propylamine have not been investigated in animals exposed by natural routes. Developmental studies in animals by natural routes of exposure would provide information on possible developmentally toxic effects, including transplacental carcinogenicity, that might be relevant to humans. These studies should include postnatal evaluations for neonatal mortality, as well as for tumor incidence in adult animals.

Immunotoxicity. Histological examination of organs and tissues of the immunological system from animals exposed in subchronic and chronic studies would provide relevant information as immunotoxicity data for N-nitrosodi-n-propylamine are not available. Specific immunotoxicity tests or a battery of immunotoxicity tests would provide a better assessment of possible

immunotoxic effects. Sensitization tests in animals might provide information on whether there is likely to be an allergic response to N-nitrosodi-n-propylamine. Although the low molecular weight of N-nitrosodi-n-propylamine would probably preclude activity as an antigen by itself, it is possible that a higher molecular weight moiety resulting from alkylation of proteins by N-nitrosodi-n-propylamine could produce an allergic response.

Neurotoxicity. Information on the neurotoxicity of N-nitrosodi-n-propylamine is not available. A battery of tests for neurotoxicity would provide further information of neurotoxicity in animals, which then might be related to possible neurotoxic effects in humans.

Epidemiological and Human Dosimetry Studies. Health effects of N-nitrosodi-n-propylamine have not been described in humans. Effects in treated animals, however, include hepatotoxicity and cancer. As discussed in Chapter 5, the potential for environmental exposure to N-nitrosodi-n-propylamine is very low, and segments of the general population with potentially high or specific exposure to N-nitrosodi-n-propylamine have not been identified. N-nitrosodi-n-propylamine has been detected in rubber manufacturing facilities but concentrations are low and exposure is complicated by the presence of other nitrosamines and additional chemicals.

If N-nitrosodi-n-propylamine or its metabolites in urine can be correlated with exposure in humans, it may be possible to monitor humans for exposure. If toxic effects of N-nitrosodi-n-propylamine are identified in humans, it may then be possible to correlate urinary levels of N-nitrosodin-propylamine or one its metabolites with systemic effects.

Biomarkers of Disease. No disease states in humans produced by exposure to N-nitrosodi-n-propylamine are known. If epidemiological studies are conducted that correlate exposure with diseases, it may be possible to identify subtle changes, such as altered blood chemistry indices, associated with a particular disease state.

Disease Registries. Diseases in humans produced by exposure to N-nitrosodi-n-propylamine are not known. If epidemiological studies identify particular diseases produced by N-nitrosodi-n-propylamine, it may be possible to determine the number of people affected and the factors associated with identifying the disease in certain populations, such as, those with exposure to high levels near hazardous waste sites.

Bioavailability from Environmental Media. No studies were located regarding the bioavailability of N-nitrosodi-n-propylamine from environmental media. The lack of data concerning levels in human tissues and fluids does not necessarily indicate a lack of bioavailability since the monitoring literature reports that N-nitrosodi-n-propylamine is present in some foods, water, beverages and workroom air. It is, therefore, important to determine if N-nitrosodi-n-propylamine can be absorbed by humans from

environmental samples. An understanding of the bioavailability of N-nitrosodi-n-propylamine from environmental media may be obtained by studying the biological fluids of individuals exposed to N-nitrosodi-n-propylamine in the workplace or through the ingestion of N-nitrosodi-n-propylamine- containing foods and beverages such as cheeses, cured meats, and whiskey. Limited information is available regarding absorption parameters of N-nitrosodi-n-propylamine in experimental animals. However, one could assume, based on data obtained with other nitrosamines, that N-nitrosodi-n-propylamine would be readily absorbed from the gastrointestinal tract if ingested from contaminated soil.

Food Chain Bioaccumulation. No studies were available concerning food chain bioaccumulation of N-nitrosodi-n-propylamine from environmental media. The monitoring literature indicates that N-nitrosodi-n-propylamine has been detected in samples of cooked fish and meat; however, occurrence of N-nitrosodi-n-propylamine in these samples was not the result of bioaccumulation, but was the result of formation resulting from preservation and/or cooking. Various studies have also shown that N-nitrosamines, such as N-nitrosodi-n-propylamine, form in the gastrointestinal tract during digestion of foods containing secondary amines. Estimation techniques have been used to determine that N-nitrosodi-n-propylamine would not bioaccumulate in lipids of fish (see Section 5.3.1, Transport and Partitioning). Based on this limited amount of information it is speculated that human exposure to N-nitrosodi-n-propylamine through diet is not the result of food chain bioaccumulation. Monitoring for the accumulation of N-nitrosodi-n-propylamine in organisms from several trophic levels could be used to support this conclusion.

Absorption, Distribution, Metabolism, Excretion. The general metabolic pathways of N-nitrosodi-n-propylamine in animals have been identified, but the relative contribution of the pathways in vivo, particularly following exposure by natural routes, is inadequately characterized. The identity of the alkylating agent(s) associated with carcinogenesis is unclear. Information is available regarding absorption and distribution of N-nitrosodi-n-propylamine. Evidence from other nitrosamines indicates that a number of factors (e.g., species, route of exposure, dosing schedule) appear to determine the organ specificity and the severity of the effects induced by these compounds. Therefore, to fully characterize the pharmacokinetics of N-nitrosodi-n-propylamine, studies of absorption, distribution, metabolism, and excretion in animals following exposure by all three routes are needed.

Comparative Toxicokinetics. The toxic and carcinogenic effects of N-nitrosodi-n-propylamine are attributable to activity of metabolites but no data are available to determine if there are quantitative differences in metabolism among species. Information from other nitrosamines suggests that there are species-characteristic tumors induced by nitrosamines. This seems to be the reflection of differences in metabolic activities (and also repair mechanisms) existing among animal species; therefore, caution must be

exercised when extrapolating possible effects in humans. Although toxicity and carcinogenicity of N-nitrosodi-n-propylamine has been demonstrated in rodents and monkeys, the animal species that serves as the best model for extrapolating results to humans may be difficult to identify.

2.9.3 On-going Studies

No on-going studies of N-nitrosodi-n-propylamine were identified.