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*-nitrosodiphenylamine. 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*-nitrosodiphenylamine, 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 relevant inhalation or dermal data were identified for *N*-nitrosodiphenylamine.

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*-nitrosodiphenylamine 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.

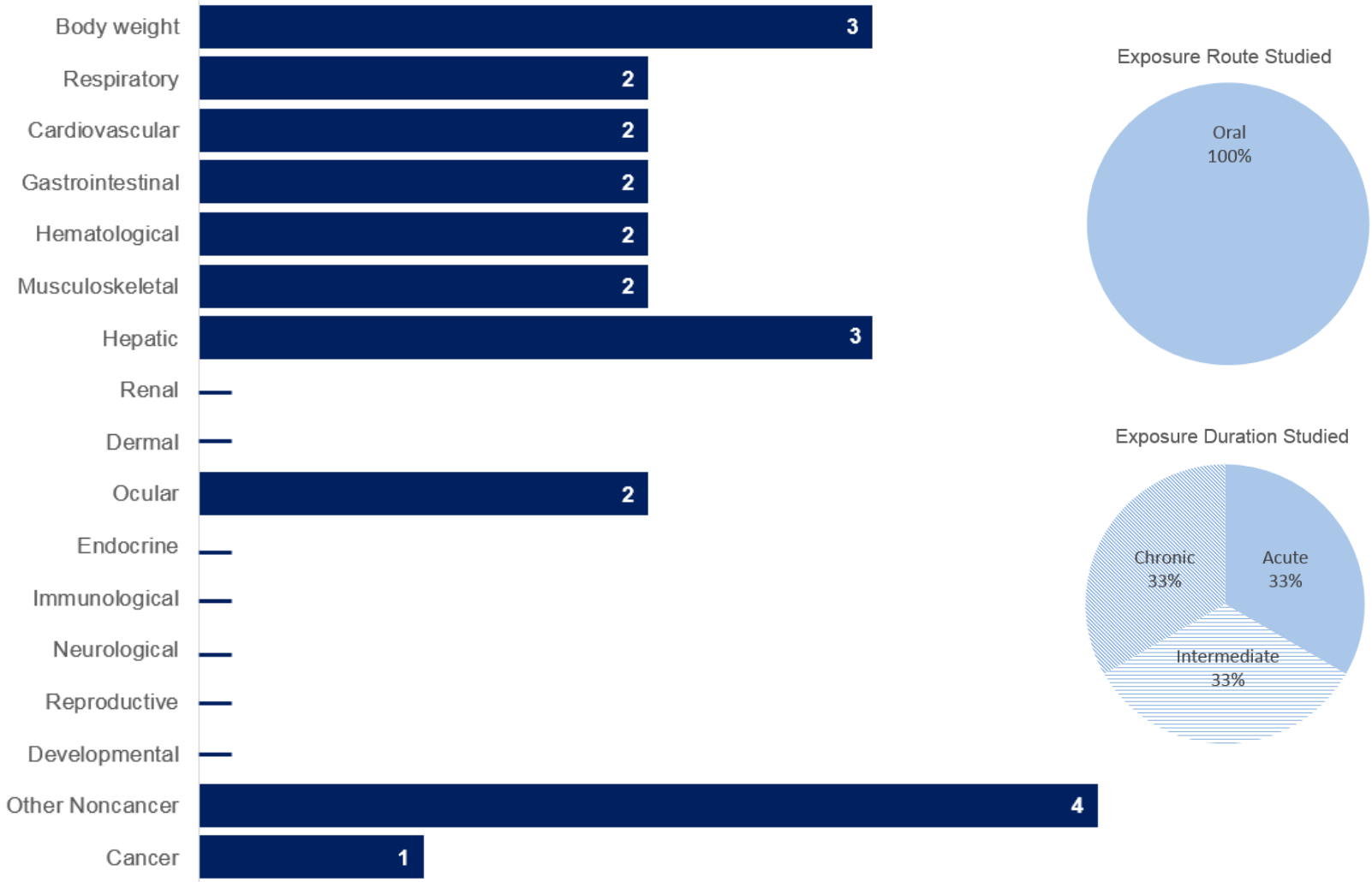
The health effects of *N*-nitrosodiphenylamine have been evaluated only in animal studies. As illustrated in Figure 2-1, all of the health effects data come from a limited number of oral exposure studies that examined the effects of *N*-nitrosodiphenylamine in rats and mice. One chronic-duration study that examined the major organs and tissues of rats and mice provided most of the information. The most examined endpoint was the urinary bladder.

The animal studies suggest one sensitive target of *N*-nitrosodiphenylamine toxicity:

- **Other Noncancer Endpoints (Urinary Bladder):** Urinary bladder effects were reported in rats and mice. In rats, there was evidence of urinary bladder alterations already after 2 weeks of exposure. After lifetime exposure, urinary lesions in rats developed into bladder carcinoma. No inference to human health can be made based on the limited animal data available.

Figure 2-1. Overview of the Number of Studies Examining *N*-Nitrosodiphenylamine Health Effects

Most studies examined the potential body weight, hepatic, and other noncancer effects of *N*-nitrosodiphenylamine
All studies evaluated health effects in **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 23 studies include those finding no effect. Most studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to *N*-Nitrosodiphenylamine – 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 (Fischer 344, F)	5 days to 2 weeks (F)	0, 15, 60, 123, 183, 251	HP, OW	Other noncancer (urinary bladder)	123	183		Increased relative urinary bladder weight (44% after 5 days and 28% after 2 weeks); histopathologic changes in the bladder: increased mitosis (6/10 after 5 days and 2 weeks), infiltrate-mixed cells (10/10 after 5 days, and diffuse hyperplasia (5/10 after 2 weeks)
Dodd et al. 2013									
2	Mouse (Swiss Webster) 10 M	4 days 1 time/day (GO)	0, 350	HP	Hepatic	350			No histopathological alterations in the liver
Nishie et al. 1972									
INTERMEDIATE EXPOSURE									
3	Rat (Fischer-344, F)	4–13 weeks (F)	0, 15, 60, 123, 183, 251	HP, OW	Other noncancer (urinary bladder)	60	123		Histopathologic changes in the bladder: infiltrate-mixed cells (6/10 after 4 weeks, 8/10 after 13 weeks) and diffuse hyperplasia (7/10 after 4 weeks, 9/10 after 13 weeks)
Dodd et al. 2013									
4	Rat F344 5 M, 5 F	8–11 weeks 7 days/week ad lib (F)	M: 0, 50, 100, 150, 200, 300, 400, 500 F: 0, 200, 400, 800, 1,200, 1,600, 2,300	BW, LE	Death			800F	2/5 died
					Bd Wt	150M	200M		>10% reduction in body weight
NCI 1979									

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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
CHRONIC EXPOSURE									
5	Rat F344 20–50 M, 20–50 F	100 weeks 7 days/week (F)	0, 50, 200	BW, HP, LE	Death			200	30% mortality in females
					Bd Wt		50		>10% reduction in body weight
					Resp	200			
					Cardio	200			
					Gastro	200			
					Hemato	200			
					Musc/skel	200			
					Hepatic	200			
					Ocular		50 F		Corneal opacity in females at 50 mg/kg/day and males at 200 mg/kg/day
					Other noncancer (urinary bladder)		50	200	Bladder epithelial hyperplasia at 50 mg/kg; squamous metaplasia at 200 mg/kg
					Cancer			200	CEL – bladder tumors
Cardy et al. 1979; NCI 1979									
6	Mouse B6C3F1 20–50 M, 20–50 F	98–101 weeks 7 days/week (F)	M: 0, 1,300, 2,600 F: 0, 301, 711	BW, HP, LE	Death			711	38% mortality in females
					Bd Wt			301 F	Body weight reduced by approximately 40% in females and 15% in males
					Resp	2,600			
					Cardio	2,600			
					Gastro	2,600			
					Hemato	2,600			
					Musc/skel	2,600			

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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
					Hepatic	2,600			
					Ocular	2,600			
					Other noncancer (urinary bladder)		301 (F)		Inflammation of bladder and bladder epithelial hyperplasia in females at 301 mg/kg/day and in males at 1,300 mg/kg/day
Cardy et al. 1979; NCI 1979									

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

ad lib = ad libitum; Bd Wt or BW = body weight; Cardio – cardiovascular; CEL = Cancer Effect Level; (F) = food; F = female(s); Gastro = gastrointestinal; (GO) = gavage in oil; Hemato = hematological; HP = histopathology; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; OW = organ weight; Resp = respiratory

Figure 2-2. Levels of Significant Exposure to *N*-Nitrosodiphenylamine – Oral

Acute (≤ 14 days)

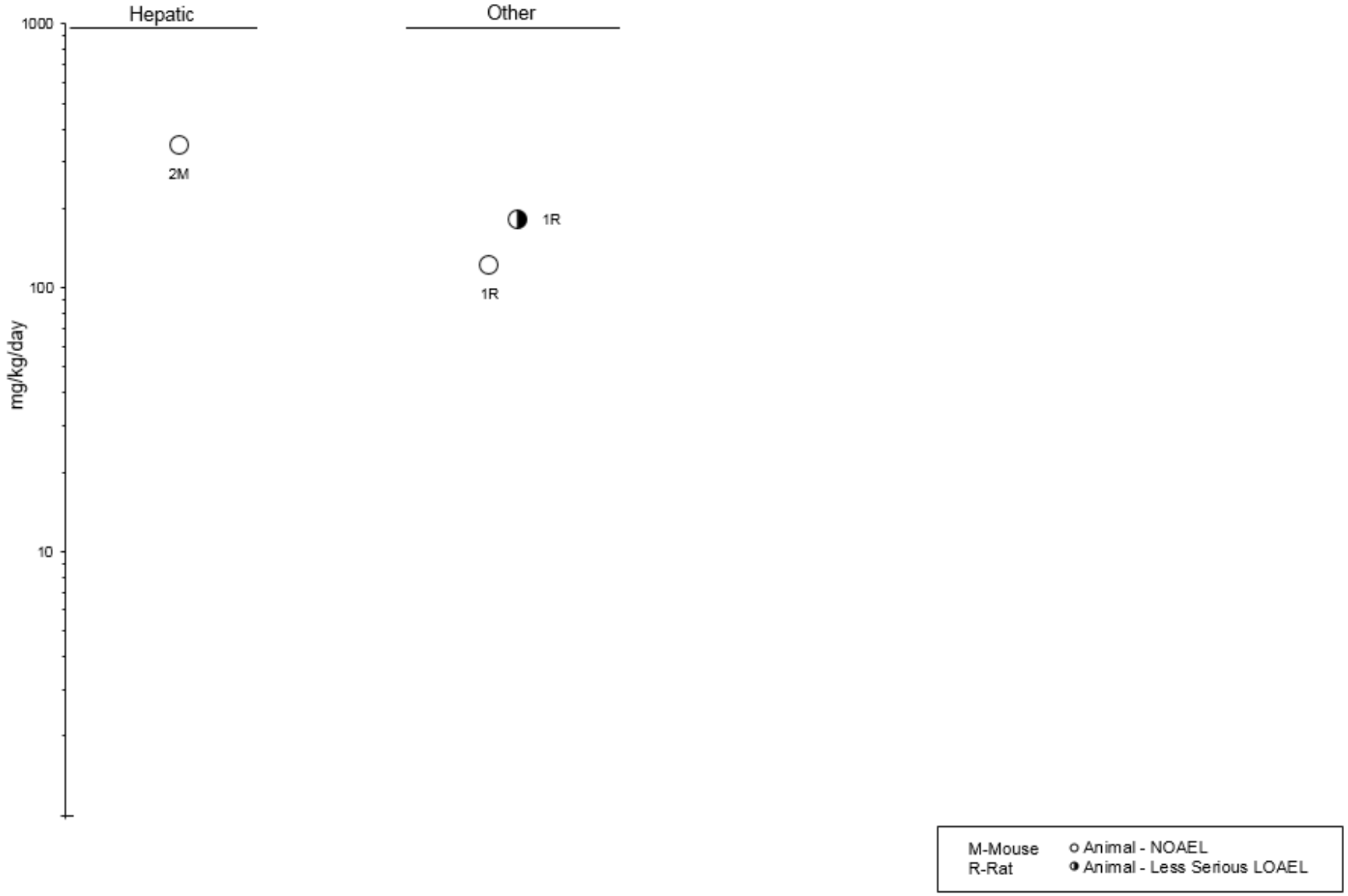


Figure 2-2. Levels of Significant Exposure to *N*-Nitrosodiphenylamine – Oral
Intermediate (15-364 days)

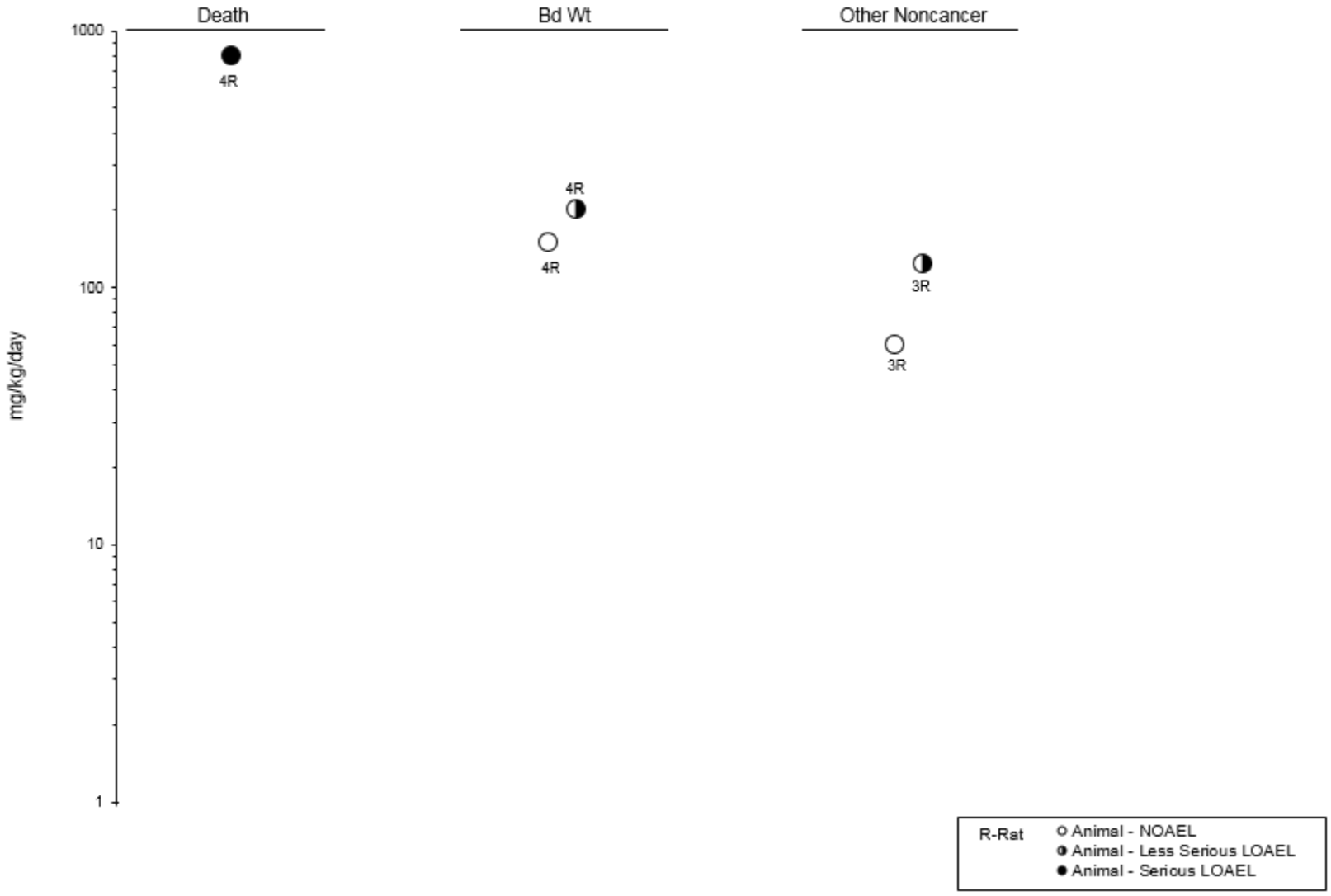
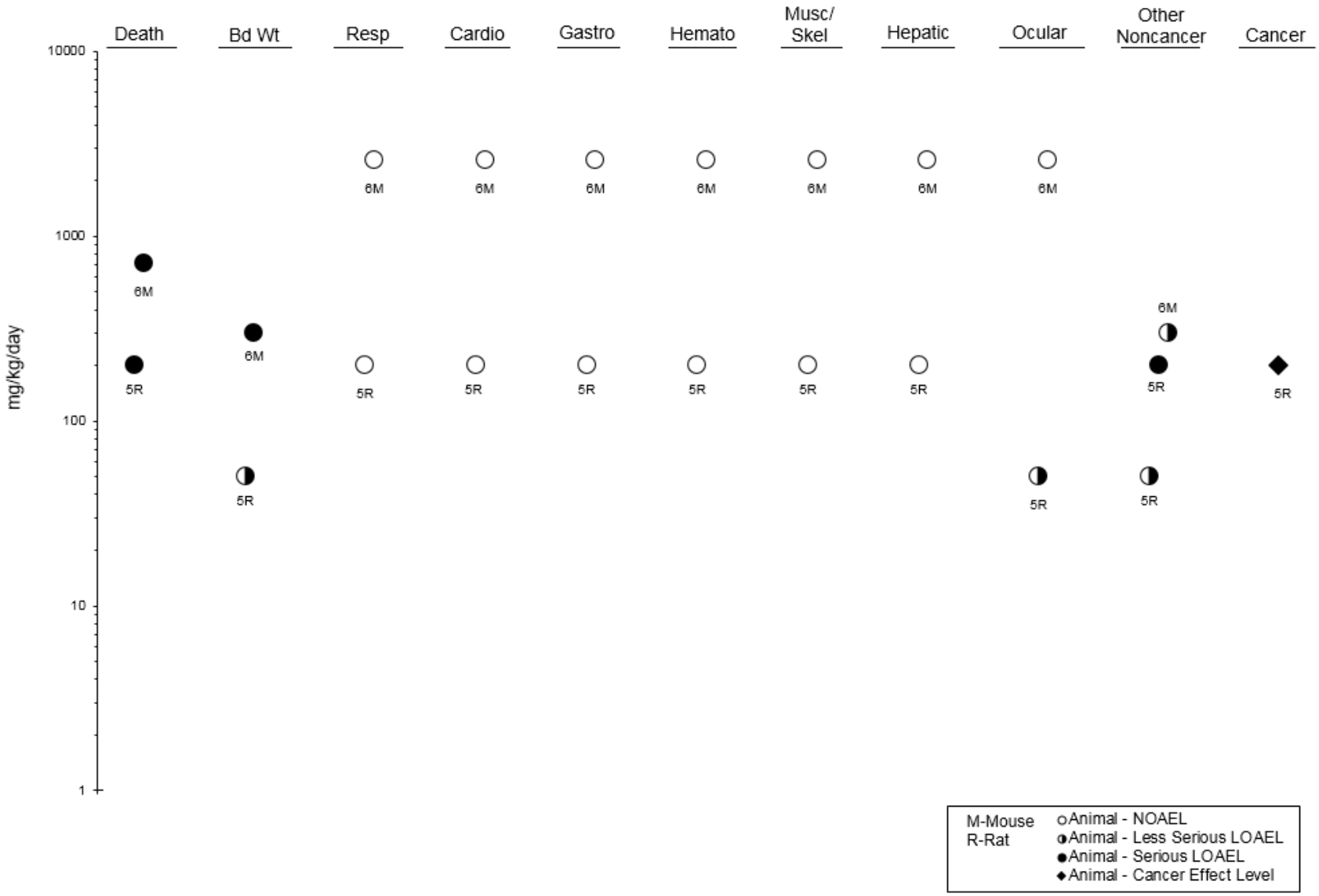


Figure 2-2. Levels of Significant Exposure to *N*-Nitrosodiphenylamine – Oral

Chronic (≥365 days)



2.2 DEATH

No studies were located regarding death in humans or animals after inhalation or dermal exposure to *N*-nitrosodiphenylamine, or in humans following oral exposure to this substance.

Only two studies on the acute oral toxicity of *N*-nitrosodiphenylamine in animals were located. Acute oral LD_{50} values of 3,000 and 3,850 mg/kg were determined for rats (Druckrey et al. 1967) and mice (Zhilova and Kasparov 1966), respectively. However, details on the methodology for these experiments were limited and detailed data were not presented.

Data from an intermediate-duration, range-finding study provide lethality data for intermediate exposure (NCI 1979). Groups of five Fischer-344 rats of each sex and five B6C3F1 mice of each sex were used in these studies. Male rats were fed diets containing 0–500 mg/kg/day of *N*-nitrosodiphenylamine for 11 weeks, and female rats were fed diets containing 0–2,300 mg/kg/day for 8 weeks. No deaths occurred in exposed male rats or in female rats given doses of <800 mg/kg/day (NOAEL of 500 mg/kg/day for male rats and 400 mg/kg/day for female rats). Two of five female rats died at 800 mg/kg/day (a LOAEL), and mortality was 100% at dietary levels of >800 mg/kg/day. In another intermediate-duration study, *N*-nitrosodiphenylamine in an aqueous methylcellulose vehicle was administered by gavage to 25 male Wistar rats at a dose of 3.1 mg/kg/day, 5 days/week, for 45 weeks (Argus and Hoch-Ligeti 1961). All rats survived until termination of the study at 53 weeks. This study provides limited information since no control groups were used and only one concentration was tested. The doses of *N*-nitrosodiphenylamine incorporated into the diet of male and female mice ranged from 0 to 5,980 mg/kg/day for 8 weeks (NCI 1979). All mice survived at all dietary levels including the highest tested. These data indicate that rats are more sensitive to the lethal effects of *N*-nitrosodiphenylamine than are mice since the dose that produced 100% mortality in rats had no effect on survival in mice.

Decreased survival was observed in rats and mice chronically exposed to *N*-nitrosodiphenylamine in their diet for 98–101 weeks (Cardy et al. 1979; NCI 1979). As in the intermediate-duration study, rats were found to be more sensitive to the lethal effects of the chemical than mice. The females of both species were more sensitive to the lethal effects of chronic exposure to *N*-nitrosodiphenylamine than the males. Fischer-344 rats of both sexes were fed diets that contained 50 or 200 mg/kg/day of *N*-nitrosodiphenylamine for 101 weeks. Male B6C3F1 mice were fed diets that contained 1,300 or 2,600 mg/kg/day for 101 weeks. Female B6C3F1 mice were initially fed diets containing 650 or 1,300 mg/kg/day, but these were reduced to 130 and 520 mg/kg/day at 38 weeks because of the drastic reduction in body weight

experienced at the higher doses. The reduced doses were continued for 60 additional weeks. The time-weighted average (TWA) concentrations for female mice over the 98 total weeks of the experiment were calculated to be 301 and 711 mg/kg/day. There were no significant treatment-related effects on survival in the male rats or male mice (NOAELs of 200 and 2,600 mg/kg/day for male rats and male mice, respectively). Survival was dose-related in the female rats, with a marginal reduction in survival at 50 mg/kg/day (NOAEL) and a more marked reduction at 200 mg/kg/day (LOAEL). In female mice, there was no dose-related survival trend; however, survival in the high-dose group was greatly reduced (LOAEL of 711 mg/kg/day) compared with that in low-dose (NOAEL of 30 mg/kg/day) and control groups.

2.3 BODY WEIGHT

In an intermediate-duration, range-finding study, rats showed a decrease in body weight of >l0% at doses of 200 mg/kg/day or more in their food (NCI 1979). Mean body weight in male rats was 12% less than the controls at 200 mg/kg/day and 16% less than the controls at the high dose (500 mg/kg/day). Mean body weight in female rats was 14% less than in the control group at the lowest dose (200 mg/kg/day) and was 37% less than the control group at the highest dose (800 mg/kg/day) at which animals survived (only two of five survived at this dose). The decreased body weight may not be indicative of an adverse effect because it is not clearly related to dose, and the pathologic data do not show tissue damage. However, full evaluation of the significance of the body weight depression is precluded because of the lack of food consumption data. The LOAEL for male and female rats was 200 mg/kg/day. A NOAEL of 150 mg/kg/day was determined for male rats. Body weights in mice exposed to concentrations of 0–5,980 mg/kg/day for 8 weeks were decreased (<14% depression) in a sporadic manner that did not appear to be related to treatment (NCI 1979).

Dose-related decreases in body weight were also reported in a chronic study (NCI 1979). Both exposure groups of rats and mice showed reduced body weight gain and reduced terminal body weight compared to control groups. A LOAEL of 50 mg/kg/day was determined for male and female rats. LOAELs of 301 and 1,300 mg/kg/day for reduced body weight were determined for female and male mice, respectively.

2.4 RESPIRATORY

No studies were located regarding respiratory effects in humans after inhalation exposure to *N*-nitroso-diphenylamine.

Rats exposed to 350-400 mg/m^3 Vulkalent A (*N*-nitrosodiphenylamine) dust for 2 hours/day were observed to have catarrhal bronchitis of the lungs (Zhilova and Kasparov 1966). Interpretation of the results of this study is not possible because of severe limitations in the experimental procedure and presentation of data. The limitations include insufficient reporting of experimental details and data, use of unspecified strains and an undefined control group, and lack of statistical analyses.

In an intermediate-duration gavage study, *N*-nitrosodiphenylamine in an aqueous methylcellulose vehicle was administered to 25 male Wistar rats at a dose of 3.1 mg/kg/day, 5 days/week, for 45 weeks (Argus and Hoch-Ligeti 1961). The rats were killed after an additional 8-week observation period. Histological examination of the lungs revealed peribronchial lymphocytic infiltration, which the authors described as common in older rats. Squamous metaplasia of the bronchial epithelium, particularly in areas of bronchiectasis, was observed in some of the lungs. Peribronchial pneumonia and emphysema were observed in rabbits administered 20 mg/kg Vulkalent A (*N*-nitrosodiphenylamine) intragastrically for 4 months (Zhilova and Kasparov 1966). It could not be determined if the respiratory effects observed in these studies were associated with *N*-nitrosodiphenylamine exposure since incidences were not reported and control groups either were not used or were not clearly defined. However, no treatment-related histological lesions of the lungs, bronchi, or trachea were observed in intermediate- and chronic-duration studies in which rats and mice were administered doses as high as 5,980 mg/kg/day for periods up to 101 weeks (NCI 1979).

2.5 CARDIOVASCULAR

No human studies have evaluated the cardiovascular toxicity of *N*-nitrosodiphenylamine. No information was located regarding cardiovascular effects in animals following inhalation or dermal exposure to *N*-nitrosodiphenylamine.

No treatment-related histological effects of the heart were reported in a chronic oral study of rats and mice administered *N*-nitrosodiphenylamine in their food (NCI 1979). No functional studies were performed that might provide data supporting the histological evidence.

2.6 GASTROINTESTINAL

No human studies have evaluated the gastrointestinal toxicity of *N*-nitrosodiphenylamine. No information was located regarding gastrointestinal effects in animals following inhalation or dermal exposure to *N*-nitrosodiphenylamine.

No treatment-related histological effects of the gastrointestinal system (esophagus, stomach, intestines, pancreas) were reported in a chronic study of rats and mice administered *N*-nitrosodiphenylamine in their food (NCI 1979). No functional studies were performed that might provide data supporting the histological evidence.

2.7 HEMATOLOGICAL

No human studies have evaluated the hematological toxicity of *N*-nitrosodiphenylamine. No information was located regarding hematological effects in animals following inhalation or dermal exposure to *N*-nitrosodiphenylamine.

No treatment-related histological effects of the bone marrow were reported in a chronic study of rats and mice administered *N*-nitrosodiphenylamine in their food (NCI 1979). Hematological tests were not conducted in the NCI (1979) study. No data were available from acute- or intermediate-duration oral exposures in animals.

2.8 MUSCULOSKELETAL

No human studies have evaluated the musculoskeletal toxicity of *N*-nitrosodiphenylamine.

No treatment-related histological effects of the musculoskeletal system were reported in a chronic oral study of rats and mice administered *N*-nitrosodiphenylamine in the food (NCI 1979). The specific tissues examined in that study were not reported. No data were available from acute- or intermediate-duration oral exposures in animals.

2.9 HEPATIC

No information was located regarding hepatic effects in humans exposed to *N*-nitrosodiphenylamine.

The limited data available indicate that the liver is not a target organ for *N*-nitrosodiphenylamine toxicity. In an acute study of hepatotoxicity (Nishie et al. 1972), mice given 350 mg/kg/day of *N*-nitrosodiphenylamine for 4 consecutive days preceding, or one dose 24 hours prior to, pentobarbital administration had effects characteristic of liver enzyme induction. These effects consisted of significantly decreased pentobarbital sleeping time and increased amounts of smooth endoplasmic reticulum among granules of glycogen in the liver cell. Electron microscopy also revealed blebs, hypertrophy, and pleomorphism of the mitochondria. A NOAEL of 350 mg/kg/day was identified for hepatic effects, since light microscopy examination did not reveal hepatic lesions.

Studies in male mice showed that administration of a single intraperitoneal dose of 20 mg/kg various *N*-nitroso compounds significantly altered the activities of both phase I and phase II metabolic enzymes in the liver (Sheweita and Mostafa 1996a, 1996b). *N*-Nitrosodiphenylamine decreased cytochrome P-450 by 54%, and arylhydrocarbon hydroxylase by 64%, and increased dimethylnitrosamine *N*-demethylase by 42%, cytochrome b_5 content by 159%, and NADPH-cytochrome *c* reductase by 57% (Sheweita and Mostafa 1996a). *N*-nitrosodiphenylamine also increased the activity of glutathione reductase by 50% and glutathione-S-transferase by 60% (Sheweita and Mostafa 1996b).

In an 8-week feeding study in rats and mice (NCI 1979), the only gross or histopathological effect reported for the liver was pigmentation of Kupffer's cells in the hepatic sinusoids in male mice that received 5,980 mg/kg/day of *N*-nitrosodiphenylamine. However, according to the tabular data presented, only female mice received this dose; the highest dose in male mice was reported as 2,860 mg/kg/day. There is no way to determine which data are incorrect. In any case, the pigmentation was presumed to reflect phagocytic activity by the Kupffer's cells. It was not considered to be adverse because only trace amounts occurred, there were no signs of toxicity or other histological alterations, and survival was not affected. In addition, no adverse liver effects were reported in rats from the same study (NCI 1979) even though rats appear more sensitive to the toxic effects of *N*-nitrosodiphenylamine than mice. Fatty and granular degeneration of the liver was reported in rabbits given 20 mg/kg Vulkalent A (*N*-nitrosodiphenylamine) for 4 months (Zhilova and Kasparov 1966). The limitations of this study are described in the discussion of renal effects in Section 2.10.

Chronic studies conducted by NCI (1979) revealed no treatment-related histological effects on the livers of exposed rats and mice. Only histological data are available and no studies of function, which might have revealed more subtle changes, were performed.

No data were available from inhalation or dermal exposure studies in animals.

2.10 RENAL

No information was located regarding renal effects in humans exposed to *N*-nitrosodiphenylamine. No data were located regarding effects in animals following inhalation or dermal exposure to *N*-nitroso-diphenylamine.

In an intermediate-duration gavage study, *N*-nitrosodiphenylamine in an aqueous methylcellulose vehicle was administered to 25 male Wistar rats at a dose of 3.1 mg/kg/day, 5 days/week, for 45 weeks (Argus and Hoch-Ligeti 1961). The rats were killed after an additional 8-week observation period. Histological examination of the kidneys revealed albuminous precipitation in the tubules of "many" kidneys. The significance of this finding in the kidneys is uncertain because incidences were not reported and control groups were not included. Albuminous degeneration of the epithelium of the kidneys was also observed in rabbits administered 20 mg/kg for 4 months (the frequency of administration was not specified) (Zhilova and Kasparov 1966). This experiment was severely limited because the strains used were not specified, the nature of control groups was uncertain, there were no statistical analyses of data, information on critical experimental details was lacking, and no quantitative data were presented.

2.11 DERMAL

No data were located regarding dermal effects in humans following dermal exposure to *N*-nitroso-diphenylamine or in animals following inhalation or oral exposure to *N*-nitrosodiphenylamine.

A single dermal study was located in which mice had 0.1 mL of a 0.1% solution of *N*-nitrosodiphenyl-amine painted on the intrascapular region once per week for 20 weeks (Iversen 1980). The investigator reported that all painted animals had small skin ulcerations and scarring. However, the significance of the results cannot be determined because it was not clear if these data included the control animals painted with the acetone solvent or only the experimental animals. Another limitation of the experiment is the use of only one dose.

2.12 OCULAR

No data were located regarding ocular effects in humans following direct contact of the eye with *N*-nitrosodiphenylamine or following oral exposure to *N*-nitrosodiphenylamine in animals following inhalation or oral exposure to *N*-nitrosodiphenylamine.

Following chronic exposure to *N*-nitrosodiphenylamine, grossly observable corneal opacity occurred at higher incidences in the high-dose male rats (15/50) and low-dose female rats (16/50) than in the corresponding control males (0/20) and control females (1/20) (NCI 1979). While the investigators concluded that this effect may have been related to treatment, the results should be viewed with caution. Incidences in the low-dose males and high-dose females were not reported, and no histopathological findings were recorded for the cornea.

2.13 ENDOCRINE

No data were located regarding endocrine effects in humans following exposure to *N*-nitrosodiphenylamine or in animals following inhalation or dermal exposure to *N*-nitrosodiphenylamine.

No histopathological lesions were observed in the salivary glands, pituitary, adrenals, or thyroid of rats or mice chronically exposed to *N*-nitrosodiphenylamine in their food (NCI 1979).

N-Nitrosodiphenylamine did not exhibit estrogenic properties in an *in vitro* assay in yeast (Nishihara et al. 2000). However, in a similar assay, *N*-nitrosodiphenylamine showed anti-androgenic activity by competitively binding to the androgen receptor against 5α-dihydrotestosterone and decreasing the level of androgen receptor protein (Hari et al. 2006).

2.14 IMMUNOLOGICAL

No studies were located regarding immunological effects in humans after exposure to *N*-nitrosodiphenylamine.

Reduced phagocytic activity of the leukocytes was reported in rats exposed to 350–400 mg/m^3 Vulkalent A (*N*-nitrosodiphenylamine) dust for 2 hours/day for 20 days (Zhilova and Kasparov 1966). Interpretation of these results is not possible because of severe limitations in the experimental procedure. These limitations are discussed in Section 2.4. No treatment-related histological effects of the

immunological system (spleen, lymph nodes, thymus) were reported in a chronic study of rats and mice administered *N*-nitrosodiphenylamine in their food (NCI 1979). No functional studies were performed that might provide data supporting the histological evidence.

2.15 NEUROLOGICAL

No studies were located regarding neurological effects in humans after exposure to *N*-nitrosodiphenylamine.

Lengthening of the chronaxie of the entensors of the rear extremities was reported in rats exposed to 350–400 mg/m^3 Vulkalent A (*N*-nitrosodiphenylamine) dust for 2 hours/day for 20 days (Zhilova and Kasparov 1966). Interpretation of these results is not possible because of severe limitations in the experimental procedure. These limitations are discussed in Section 2.4. No treatment-related histological effects were reported in the brains of rats and mice chronically exposed to *N*-nitrosodiphenylamine in their food (NCI 1979). No functional studies were performed that might provide data supporting the histological evidence.

2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans following exposure to *N*-nitrosodiphenylamine.

No treatment-related histological effects of the testes, prostate, uterus, or ovaries were reported in chronic studies that might provide data supporting the histological evidence study of rats and mice administered *N*-nitrosodiphenylamine in their food (NCI 1979). No functional studies were performed that might provide data supporting the histological evidence. No data are available in animals following inhalation or dermal exposure to *N*-nitrosodiphenylamine.

2.17 DEVELOPMENTAL

No data are available regarding developmental effects in humans or in animals following exposure to *N*-nitrosodiphenylamine.

2.18 OTHER NONCANCER

Data from oral studies in rats and mice indicate that the bladder is a target organ for chronic oral exposure to *N*-nitrosodiphenylamine. Exposure of male rats to 183 mg/kg/day *N*-nitrosodiphenylamine in the food for 2 weeks significantly increased the incidence of mixed cell infiltrate and diffuse hyperplasia in the transitional epithelium of the urinary bladder; no significant changes were reported at 123 mg/kg/day (Dodd et al. 2013). Continued exposure for up to 13 weeks resulted in similar lesions in rats exposed to 123 mg/kg/day *N*-nitrosodiphenylamine, but not in rats dosed with 60 mg/kg/day (Dodd et al. 2013). Absolute and relative bladder weight were significantly increased in rats dosed with ≥183 mg/kg/day, but no significant changes were reported at 123 mg/kg/day. Epithelial hyperplasia of the urinary bladder increased in frequency with dose in both male and female rats given doses of 50 and 200 mg/kg/day *N*-nitrosodiphenylamine in their diet for approximately 2 years (Cardy et al. 1979; NCI 1979). Squamous metaplasia of the bladder, a more serious lesion, occurred at low incidences and only in the high-dose animals. It is likely that the bladder hyperplasia and metaplasia were preneoplastic effects since transitional cell carcinoma also occurred in the high-dose rats (see Section 2.19).

Effects on the bladder from chronic exposure to *N*-nitrosodiphenylamine also occurred in mice (Cardy et al. 1979; NCI 1979). Male mice received 1,300 or 2,600 mg/kg/day *N*-nitrosodiphenylamine in the diet for 101 weeks, and females received 301 or 711 mg/kg/day (TWA concentrations) in the diet for 98 weeks (see Section 2.2 for details of female dosing). Incidences of submucosal inflammation of the urinary bladder in the control, low-dose, and high-dose groups were 0/18, 12/49, and 31/46, respectively, in the males and 0/18, 31/47, and 30/38, respectively, in the females. The inflammatory response was associated with connective tissue degeneration in the submucosa. Epithelial hyperplasia of the bladder in the control, low-dose, and high-dose groups occurred in 0/18, 2/49, and 7/46 males, respectively, and 0/18, 3/47, and 6/38 females respectively, but increased incidences of bladder neoplasms were not statistically significant. LOAELs of 1,300 and 301 mg/kg/day were identified for inflammation of the bladder submucosa in males and females, respectively.

2.19 CANCER

No information was located regarding cancer effects in humans following oral or dermal exposure to *N*-nitrosodiphenylamine or in animals following inhalation exposure.

Straif et al. (2000) published the results of a study of cancer occurrence among German workers exposed to nitrosamines (*N*-nitrosodiphenylamine among them) in the rubber industry. The cohort consisted of

8,933 workers hired after January 1959 and followed up for mortality from January 1st 1981 until December 31st 1991. *N*-Nitrosodiphenylamine had been used as a retarder until early 1980, but specific exposure concentrations to this chemical in the air were not available. Hazard rate ratios calculated for high exposure (>15 µg/m^3) to total nitrosamines showed significantly elevated risks for cancers of the esophagus and oral cavity and pharynx compared to the upper boundary for a low exposure category (<2.5 µg/m^3). In this study, the specific role of *N*-nitrosodiphenylamine, if any, cannot be determined.

One intermediate-duration study was located in which 25 male Wistar rats received *N*-nitrosodiphenylamine in an aqueous methylcellulose vehicle by gavage at a dose of 11.63 mg/kg/day, 5 days/week, for 45 weeks (Argus and Hoch-Ligeti 1961). No tumors were found in the treated animals. Histological examinations were limited to the liver, spleen, kidneys, lungs, and organs with gross abnormalities.

Rats were more sensitive than mice to the carcinogenic effects of *N*-nitrosodiphenylamine according to an NCI (1979) study. *N*-Nitrosodiphenylamine was administered in the diet to Fischer-344 rats and B6C3F1 mice (50/sex/strain) with the matched control groups consisting of 20 untreated rats and mice of each sex (Cardy et al. 1979; NCI 1979). Comprehensive gross and histopathological examinations were conducted on animals that died during the study and on all animals that survived to the end of the study. The highest incidence of tumors was found in the urinary bladder of rats.

Rats received 50 and 200 mg/kg/day *N*-nitrosodiphenylamine in the diet for 100 weeks (Cardy et al. 1979; NCI 1979). A significant increase ($p<0.001$) in the incidence of transitional cell carcinomas in the urinary bladder occurred in rats receiving the highest dose (an increase of 38% in males and 86% in females) compared to the controls. An increase in fibromas of the integumentary system (i.e., subcutis and skin) occurred in male rats, but this increase was not statistically significant. The investigators noted that the occurrence of fibromas was probably associated with treatment because integumentary system fibromas were rare in historical controls at the same laboratory. The results of the study are sufficient to conclude that *N*-nitrosodiphenylamine is carcinogenic in male and female Fischer-344 rats. The EPA (IRIS 2002) has classified *N*-nitrosodiphenylamine in Group B2, probable human carcinogen. IARC has classified *N*-nitrosodiphenylamine in Group 3, not classifiable as to its carcinogenicity to humans (IARC 2017).

An earlier study reported negative results in rats; however, uncertainties are associated with the study because the bladders were not routinely examined, smaller groups of rats were studied, and doses were lower than those provided by the NCI (1979) dietary levels. *N*-Nitrosodiphenylamine was administered

to 20 BD rats of unspecified sex in drinking water that provided a daily dose of 120 mg/kg and a total dose of 65,000 mg/kg (Druckrey et al. 1967). Histopathologic examinations consisting of gross evaluation of the liver, brain, and unspecified organs were conducted after 700 days, but there was no evidence of tumors in the treated animals.

Male B6C3F1 mice were fed 1,300 or 2,600 mg/kg/day *N*-nitrosodiphenylamine for 101 weeks (Cardy et al. 1979; NCI 1979). Female $B6C3F_1$ mice initially received 650 or 1,300 mg/kg/day for 38 weeks, but because of an excessive reduction in mean weight gain, dosing was discontinued for 3 weeks and then resumed at 130 or 520 mg/kg/day for 60 weeks. TWAs of 301 and 711 mg/kg/day were determined for the low- and high-dose females, respectively. Transitional cell carcinoma of the bladder was reported in a low-dose male and female, as well as transitional cell papilloma in a high-dose male. However, there was no statistically significant increase in tumor incidence in the treated animals. The authors concluded that *N*-nitrosodiphenylamine was not carcinogenic in mice under the test conditions used.

B6C3F1 and B6AKF1 mice (18 sex/strain) initially received 1,000 mg/kg/day of *N*-nitrosodiphenylamine in dimethyl sulfoxide by gavage from 7 to 28 days of age, and subsequently in the diet at a concentration of 490 mg/kg/day until 81 or 83 weeks of age (Innes et al. 1969; NCI 1968). Negative and positive controls were tested. An increased incidence of hepatomas, of borderline statistical significance, was observed in only 6 of 18 treated B6C3F1 males. The histological examinations in this study were usually limited to the chest contents, liver, spleen, kidneys, adrenals, stomach, intestines, and genital organs. The bladder was not examined, so it is possible that results similar to those of the NCI (1979) study might have been obtained had the bladder been examined. The equivocal liver results from the early NCI (1968) study might be explained by the high percentage of liver neoplasms (30 and 20% in male and female control groups, respectively) found in all groups of B6C3F1 mice in the later NCI (1979) study. This strain of mice may have a genetic tendency towards liver lesions.

Single weekly 0.1-mL applications of a 1% solution of *N*-nitrosodiphenylamine (33 mg/kg/week) in acetone were placed on the intrascapular region of 16 male and 24 female hairless hr/hr Oslo strain mice for 20 weeks (Iversen 1980). Gross and histological examinations were performed on the lungs and palpable lesions of surviving animals (14 males, 21 females) following 80 weeks of observation. The only tumors detected were lung adenomas in three of the treated males. The study was limited because the treatment duration was short, the frequency was low, only one low exposure level was tested, histopathological examinations were limited, and control data were not available.

The mechanism by which *N*-nitrosodiphenylamine induces bladder cancer in rats has not been elucidated but it does not appear to involve direct alkylation. Instead, there is some evidence that a mechanism involving transnitrosation may play a role. Further details regarding this potential mechanism are presented below in Section 2.20

2.20 GENOTOXIC

No epidemiology or case studies were available for genotoxicity of *N*-nitrosodiphenylamine in humans. The only human data regarding the genotoxic effects of this chemical come from *in vitro* assays for deoxyribonucleic acid (DNA) damage and sister chromatid exchange. Human fibroblasts were used to test for DNA damage from metabolically activated *N*-nitrosodiphenylamine (Agrelo and Amos 1981; Martin and McDermid 1981; Snyder and Matheson 1985). Only one of the three studies produced a positive response (see Table 2-3). A positive but statistically insignificant response was noted for increased sister chromatid exchange frequency in human lymphocytes after exposure to activated *N*-nitrosodiphenylamine (Lindahl- Kiessling et al. 1989). It is difficult to draw conclusions for humans from these data. However, from these and other studies it appears that *N*-nitrosodiphenylamine is not a human clastogen.

In vivo animal studies involving mice and rats consistently show negative results for DNA damage, micronuclei, DNA synthesis inhibition, and abnormal sperm morphology (Table 2-2). A gene mutation study (wing spot test) and a recessive lethal study involving *Drosophila melanogaster* both produced negative results as well (Negishi et al. 1991; Vogel et al. 1981). However, in a host-mediated assay, a positive response for DNA damage was observed in *Escherichia coli* that were injected along with *N*-nitrosodiphenylamine into the abdomina of male *D. melanogaster* (Knasmuller et al. 1990). The most commonly tested route of exposure for these studies was intraperitoneal injection in mice (McFee et al. 1989; Salamone et al. 1981; Topham 1981; Tsuchimoto and Matter 1981). Oral exposure was tested in only three studies (Brambilla et al. 1987; Friedman and Staub 1976; McFee et al. 1989). From this information, *N*-nitrosodiphenylamine does not appear to be genotoxic to intact animal systems.

Table 2-2. Genotoxicity of *N*-Nitrosodiphenylamine *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Mammalian cells			
Rat (liver nuclei)	DNA damage	–	Brambilla et al. 1987
Mouse (bone marrow cells)	Micronucleus assay	–	McFee at al. 1989

Table 2-2. Genotoxicity of *N*-Nitrosodiphenylamine *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Mouse (bone marrow cells)	Micronucleus assay	–	Salamone et al. 1981
Mouse (bone marrow cells)	Micronucleus assay	–	Tsuchimoto and Matter 1981
Mouse (testicular cells)	DNA synthesis inhibition	–	Friedman and Staub 1976
Mouse (cauda epididymis)	Abnormal spermal morphology	–	Topham 1981
Non-mammalian cells			
Drosophilia melanogaster (wing spot test)	Gene mutation	–	Negishi et al. 1991
D. melanogaster (recessive lethal test)	Gene mutation	–	Vogel et al. 1981
Escherichia coli (host-mediated assay)	DNA damage	+	Knasmuller et al. 1990

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

Data from *in vitro* studies using prokaryotic and eukaryotic organisms and cultured mammalian cells are presented in Table 2-3. Responses were negative in the majority of the gene mutation studies. However, two *Salmonella typhimurium* assays detected gene mutations after exposure to metabolically activated *N*-nitrosodiphenylamine (Khudoley et al. 1987; Zielenska and Guttenplan 1988) and one study reported weakly positive results for gene mutation in the absence of metabolic activation (Kubo et al. 2002). *N*-Nitrosodiphenylamine exhibited no effect on mitotic crossing-over and gene conversion in *Saccharomyces cerevisiae* (Jagannath et al. 1981; Kassinova et al. 1981; Sharp and Parry 1981a). Chromosomal aberration assays for Chinese hamster fibroblasts and Don cells were inconclusive (Abe and Sasaki 1977; Ishidate and Odashima 1977). Sister chromatid exchange was unaffected in hamster ovary cells (Evans and Mitchell 1981; Perry and Thomson 1981), but a positive response for sister chromatid exchange was noted in hamster Don cells after exposure to *N*-nitrosodiphenylamine that had not been metabolically activated (Abe and Sasaki 1977). Micronuclei were not induced in Chinese hamster ovary cells either with or without the addition of metabolic activation (Phelps et al. 2002). In a cellular transformation assay in mouse BALB/c cells, a weak positive result was reported in the presence of metabolic activation (Mathews et al. 1993). Tests for DNA damage have produced mixed results among prokaryotes and fungi. Among mammalian hepatocytes, however, the results for DNA damage were positive. As mentioned previously, only one study involving cultured human fibroblasts was positive for DNA damage (Snyder and Matheson 1985).

Table 2-3. Genotoxicity of *N*-Nitrosodiphenylamine *In Vitro*

Species (test system)	Endpoint	Results		Reference
		Activation		
		With	Without	
Prokaryotic organisms				
Salmonella typhimurium (pour plate and premincubation assay)	Gene mutation	–	NA	Araki et al. 1984
S. typhimurium (Ames assay)	Gene mutation	–	–[a]	Crebelli et al. 1984
S. typhimurium (Ames assay)	Gene mutation	NA	–	Ichinotsubo et al. 1981
S. typhimurium (Ames assay)	Gene mutation	+	NA	Khudoley et al. 1987
S. typhimurium (Ames assay)	Gene mutation	–	(+)	Kubo et al. 2002
S. typhimurium (modified Ames assay)	Gene mutation	–	–	Probst et al. 1981
S. typhimurium (Ames assay)	Gene mutation	–	NA	Raineri et al. 1981
S. typhimurium (Ames assay)	Gene mutation	–	–	Wagner et al. 2012
S. typhimurium (liquid preincubation assay)	Gene mutation	+	NA	Zielenska and Guttenplan 1988
S. typhimurium (umu assay)	Gene mutation	–	NA	Degirmenci et al. 2000
Escherichia coli (pour plate and preincubation assay)	Gene mutation	–	NA	Araki et al. 1984
E. coli (reverse mutation preincubation)	Gene mutation	–	–	Matsushima et al. 1981
E. coli (modified Ames assay)	Gene mutation	–	–	Probst et al. 1981
S. typhimurium (umu gene response)	DNA damage	–	NA	Shimada et al. 1989
E. coli (differential killing test)	DNA damage	+	NA	Green 1981
E. coli (rec assay)	DNA damage	–	NA	Mamber et al. 1983
E. coli (disc diffusion and liquid suspensión assay)	DNA damage	–	NA	Rosenkranz et al. 1981
E. coli (differential killing test)	DNA damage	–	+	Tweats 1981
Bacillus subtilis (rec assay)	DNA damage	–	+	Kada 1981
Eukaryotic organisms, non-mammalian				
Schizosaccharomyces pombe (forward mutation assay)	Gene mutation	–	–	Loprieno 1981
Saccharomyces cerevisiae (rep-test)	DNA damage	NA	–	Kassinova et al. 1981
S. cerevisiae (rad assay)	DNA damage	–	+	Sharp and Parry 1981b

Table 2-3. Genotoxicity of *N*-Nitrosodiphenylamine *In Vitro*

Species (test system)	Endpoint	Results		Reference
		Activation		
		With	Without	
S. cerevisiae (ade2 locus assay)	Mitotic crossing over	–	–	Kassinova et al. 1981
S. cerevisiae (his4, trp5 assay)	Mitotic gene conversion	–	–	Sharp and Parry 1981a
S. cerevisiae (ade2, trp5 assay)	Mitotic gene conversion	–	–	Jagannath et al. 1981
Mammalian cells				
Rat (embryo cells)	Gene mutation	–	–	Mishra et al. 1978
Chinese hamster (V79 cells)	Gene mutation	–	–	Jones and Huberman 1980
Chinese hamster (V79 cells)	Gene mutation	–	–	Kuroki et al. 1977
Mouse (lymphoma cells)	Gene mutation	–	–	Clive et al. 1979
Mouse (lymphoma cells)	Gene mutation	–	–	Jotz and Mitchell 1981
Mouse (lymphoma cells)	Gene mutation	–	–	Oberly et al. 1984
Human (fibroblasts)	DNA damage	–	NA	Agrelo and Amos 1981
Human (fibroblasts)	DNA damage	–	NA	Martin and McDermid 1981
Human (fibroblasts)	DNA damage	+	NA	Snyder and Matheson 1985
Rat (hepatocytes)	DNA damage	+	NA	Althaus et al. 1982
Rat (hepatocytes)	DNA damage	+	NA	Althaus and Pitot 1983
Rat (hepatocytes)	DNA damage	+	NA	Bradley et al.1982
Rat (hepatocytes)	DNA damage	+	NA	Probst et al. 1981
Rat (hepatocytes)	DNA damage	+	NA	Sina et al. 1983
Chinese hamster (hepatocytes)	DNA damage	+	NA	McQueen et al.1983
Mouse (hepatocytes)	DNA damage	+	NA	McQueen et al.1983
Mouse (BALB/c cells)	Cellular transformation	–	(+)	Matthews et al. 1993
Phelps et al. 2002	Micronucleus assay	–	–	Phelps et al. 2002
Chinese hamster (Don cells)	Chromosomal aberrations	NA	±	Abe and Sasaki 1977
Chinese hamster (fibroblasts)	Chromosomal aberrations	NA	±	Ishidate and Odashima 1977
Human (lymphocytes)	Sister chromatid exchange	(+)	NA	Lindahl-Kiessling et al. 1989
Chinese hamster (ovary cells)	Sister chromatid exchange	–	–	Evans and Mitchell 1981
Chinese hamster (Don cells)	Sister chromatid exchange	NA	+	Abe and Sasaki 1977

Table 2-3. Genotoxicity of *N*-Nitrosodiphenylamine *In Vitro*

Species (test system)	Endpoint	Results: Activation: With	Results: Activation: Without	Reference
Chinese hamster (ovary cells)	Sister chromatid exchange	–	–	Perry and Thomson 1981

[a]A positive response was observed after *in vitro* nitrosation.

+ = positive results; (+) = weakly positive results; – = negative results; ± = inconclusive or equivocal; DNA; deoxyribonucleic acid; NA: not applicable

Most of the positive *in vitro* responses occurred in cases where exogenous metabolic activation was involved. This suggests that if *N*-nitrosodiphenylamine has genotoxic potential, the potential may arise from its metabolites. In fact, many *N*-nitroso compounds are thought to exert their mutagenic and carcinogenic effects through intermediates derived from alpha-carbon hydroxylation; these intermediates can alkylate DNA (Magee et al. 1976; Preussman and Stewart 1984; Schut and Castonguay 1984). However, since *N*-nitrosodiphenylamine is not susceptible to alpha-carbon oxidation, it presumably exerts its action by some mechanism other than direct alkylation. Some researchers have speculated that the carcinogenicity of *N*-nitrosodiphenylamine is due to transnitrosation with carcinogenic *N*-nitroso derivative(s) formation (NCI 1979; Preussmann and Stewart 1984; Raineri et al. 1981). An example of the reaction can be found in the formation of nitrosamines by nitrosation of dietary amines. This theory is supported by a *Salmonella* Ames test in which *N*-nitrosodiphenylamine was found to be mutagenic in strains TA98 and TA100 only after it was nitrosated *in vitro*; significantly positive responses were observed in systems without activation (Crebelli el al. 1984). Alternatively, transnitrosation could occur from *N*-nitrosodiphenylamine to another compound. Evidence exists for transnitrosation by *N*-nitroso-diphenylamine *in vivo*; transnitrosation from *N*-nitrosodiphenylamine to proline occurred in rats when the compounds were coadministered orally (Ohshima et al. 1982). The transnitrosation mechanism is consistent with the negative results obtained for *N*-nitrosodiphenylamine in assays for mutagenicity (with or without metabolic activation) and the positive results obtained in the NCI (1979) dietary carcinogenesis study in rats.