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 of the toxicology of *N*-nitrosodiphenylamine and a depiction of significant exposure levels associated with various adverse health effects. It contains descriptions and evaluations of studies and presents levels of significant exposure for *N*-nitrosodiphenylamine based on toxicological studies and epidemiological investigations.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the information in this section is 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 (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverseeffect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have heen 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. They should also help to determine whether or not the effects vary 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 in the tables and figures may differ depending on the user’s perspective. For example, physicians concerned with the interpretation of clinical findings. in exposed persons may be interested in levels of exposure associated with “serious” effects. Public health officials and project managers concerned with appropriate actions to take at hazardous waste 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) may be of interest to health professionals and citizens alike.

Levels of exposure associated with the carcinogenic effects of *N*-nitrosodiphenylamine are indicated in Figure 2-l. Because cancer effects could occur at lower exposure levels, the figures also show a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to $l0^{-7}$), 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 effect for each exposure duration. MRLs include adjustments to reflect human variability and extrapolation of data from laboratory animals to humans.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1989a), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

2.2.1 Inhalation Exposure

2.2.1.1 Death

No studies were located regarding death in humans or animals after inhalation exposure to *N*-nitrosodiphenylamine.

2.2.1.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, or dermal/ocular effects in humans or animals after inhalation exposure to *N*-nitrosodiphenylamine.

Respiratory Effects. No studies were located regarding respiratory effects in humans after inhalation exposure to *N*-nitrosodiphenylamine.

Rats exposed to 350-400 mg/m^3 Vulkalent A (*N*-nitrosodiphenylamine) dust for 2 hours per 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 experimenta! details and data, use of unspecified strains and an undefined control group, and lack of statistical analyses.

2.2.1.3 Immunological Effects

No studies were located regarding immunological effects in humans after inhalation 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 per 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 2.2.1.2.

2.2.1.4 Neurological Effects

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

A lengthening of the chronaxie of the extensors of the rear extremities was observed in rats exposed to 350-400 mg/m^{3}Vulkalent A (*N*-nitrosodiphenylamine) dust for 2 hours per 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 2.2.1.2.

2. HEALTH EFFECTS

No studies were located regarding the following health effects in humans or animals after inhalation exposure to *N*-nitrosodiphenylamine:

2.2.1.5 Developmental Effects
2.2.1.6 Reproductive Effects
2.2.1.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

No studies were located regarding cancer in humans or animals after inhalation exposure to *N*-nitrosodiphenylamine.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to *N*-nitrosodiphenylamine.

Only two studies on the acute oral toxicity of *N*-nitrosodiphenylamine in animals were located. Acute oral LD_{50} values of 3,000 mg/kg 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 $B6C3F_1$ 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 per 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 iV-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 1ClCl weeks. Male $B6C3F_1$ mice were fed diets that contained 1,300 or 2,600 mg/kg/day for 101 weeks. Female $B6C3F_1$ 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.

All reliable LOAEL values for death in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.2.2 Systemic Effects

No studies were located regarding systemic effects in humans after oral exposure to *N*-nitrosodiphenylamine. The highest NOAEL values and all reliable LOAEL values for systemic effects in rats and mice following acute, intermediate, and chronic exposure are recorded in Table 2-l and plotted in Figure 2-l.

Respiratory Effects. 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 &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).

Cardiovascular Effects. No treatment-related histological effects of the heart 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.

Gastrointestinal Effects. 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.

Hematological Effects. 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). No functional studies were performed that might provide data supporting the histological evidence.

TABLE 2-1. Levels of Significant Exposure to N-Nitrosodiphenylamine - Oral

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
ACUTE EXPOSURE								
Systemic								
1	Mouse	(GO)	4 d 1x/d	Hepatic	350			Nishie et al. 1972
INTERMEDIATE EXPOSURE								
Death								
2	Rat	(F)	8-11 wk 7d/wk ad lib				800 (2/5 died)	NCI 1979
Systemic								
3	Rat	(F)	8-11 wk	Other	150	200 (>10% reduction in body weight)		NCI 1979
CHRONIC EXPOSURE								
Death								
4	Rat	(F)	100 wk 7d/wk				200 (30% mortality in females)	NCI 1979
5	Mouse	(F)	98-101 wk 7d/wk				711 (38% mortality in females)	NCI 1979

TABLE 2-1 (Continued)

Key to figure[a]	Species		Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kd/day)	Reference
Systemic								
6	Rat	(F)	100 wk 7d/wk	Resp	200			Cardy et al. 1979; NCI 1979
				Cardio	200			
				Gastro	200			
				Hemato	200			
				Musc/skel	200			
				Hepatic	200			
				Renal		50 (bladder epithelial hyperplasia)	200 (squamous metaplasia)	
				Derm/oc		200 (corneal opacity in males) 50 (corneal opacity in females)		
				Other		50 (body weight reduced >10%)		
7	Mouse	(F)	98-101 wk 7d/wk	Resp	2600			Cardy et al. 1979; NCI 1979
				Cardio	2600			
				Gastro	2600			
				Hemato	2600			
				Musc/skel	2600			
				Hepatic	2600			
				Renal		301 (inflammation of bladder and bladder epithelial hyperplasia in females) 1300 (inflammation of bladder and bladder epithelial hyperplasia in males)		
				Derm/oc	2600			
				Other		301 (body weight reduced approximately 40% in females) 1300 (body weight reduced approximately 15% in males)		

TABLE 2-1 (Continued)

Key to figure[a]	Species		Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kd/day)	Reference
Cancer								
8	Rat	(F)	100 wk 7d/wk				200 (CEL - bladder tumors)	Cardy et al. 1979

[a]The number corresponds to entries in Figure 2-1.

ad lib = ad libitum; Cardio = cardiovascular; CEL = Cancer Effect Level; d = day(s); Derm/oc = dermal/ocular; (F) = food; Gastro = gastrointestinal; (GO) = gavage in oil; Hemato = hematological; LOAEL = lowest-observed-adverse-effect level; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); x = time(s)

FIGURE 2-1. Levels of Significant Exposure to *N*-Nitrosodiphenylamine - Oral

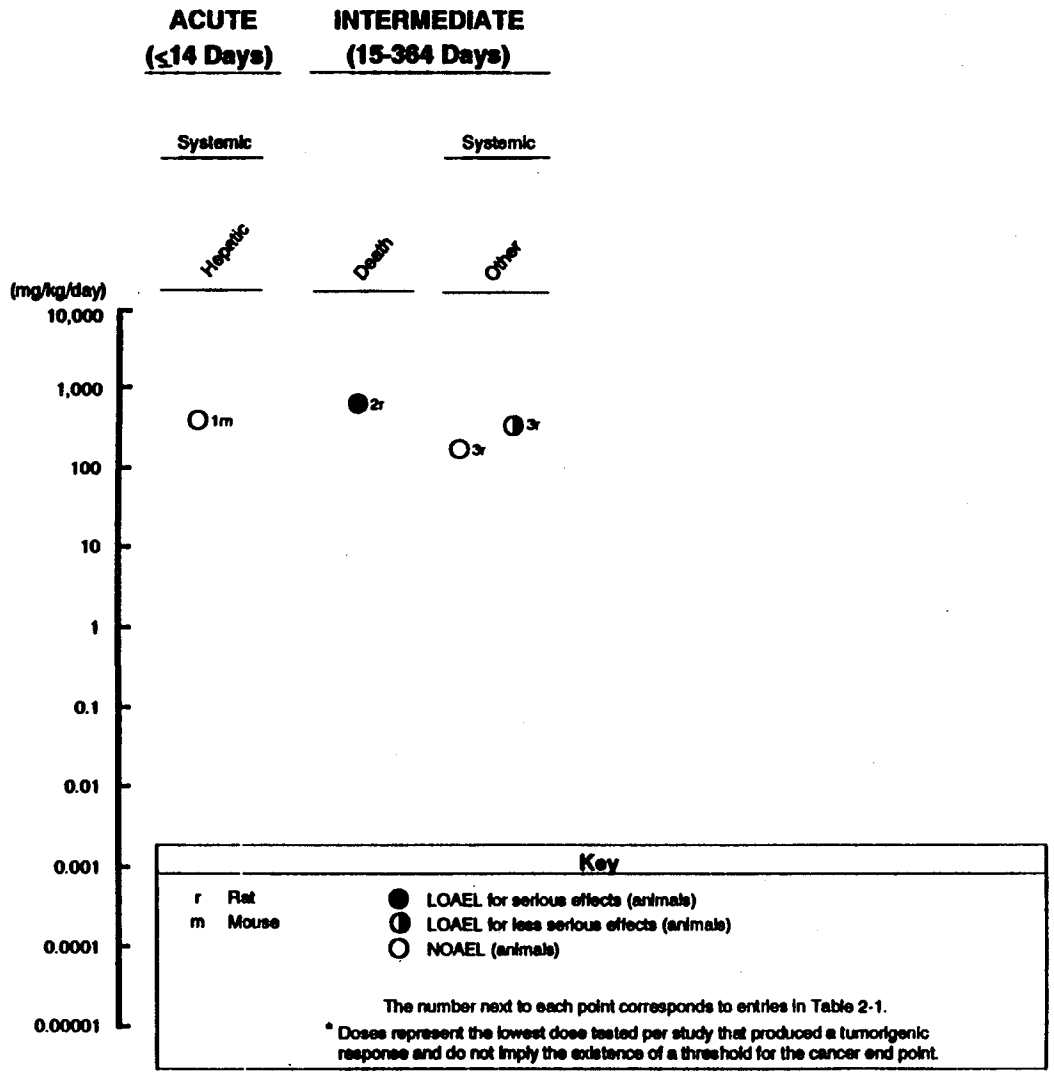
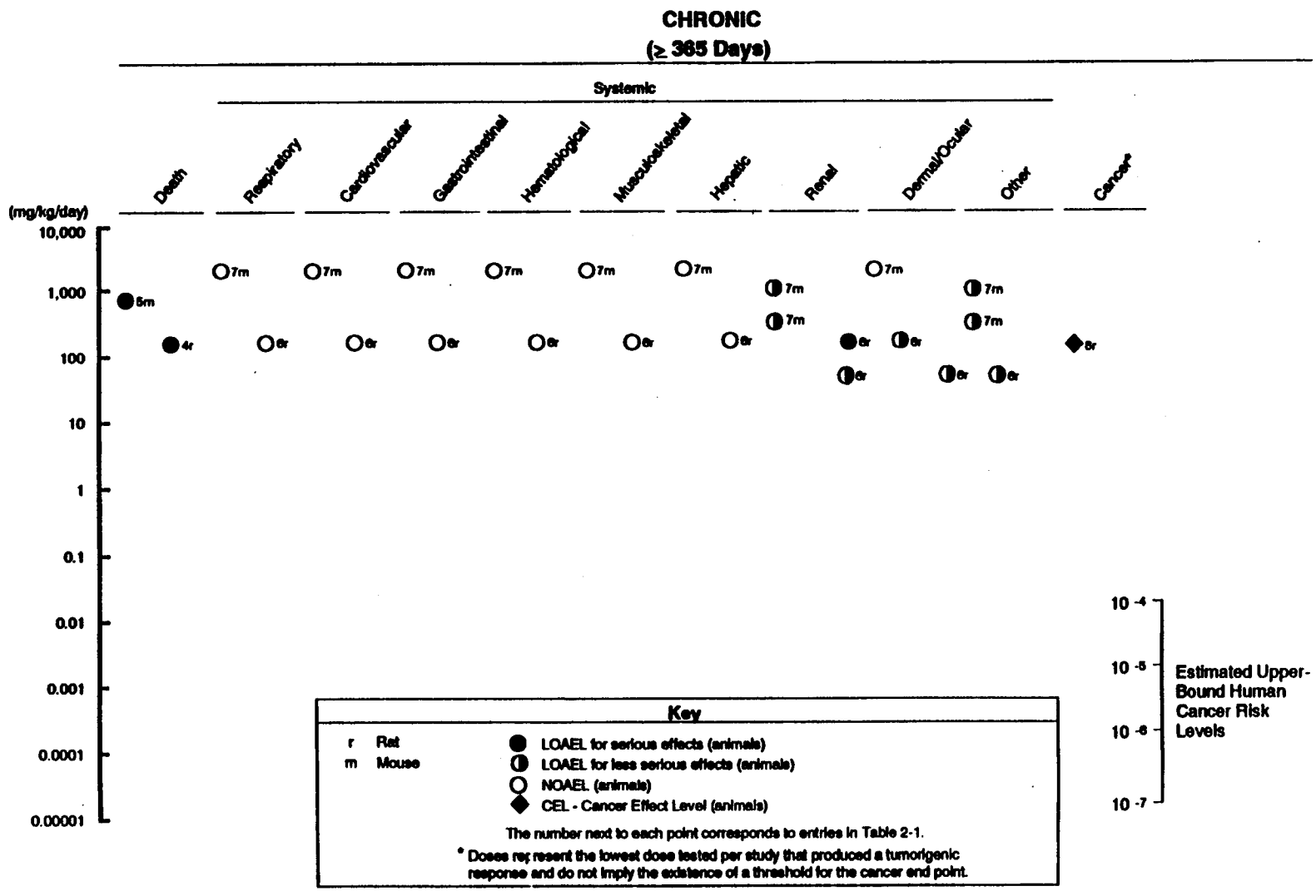


FIGURE 2-1 (Continued)



Musculoskeletal Effects. No treatment-related histological effects of the musculoskeletal system were reported in a chronic study of rats and mice administered *N*-nitrosodiphenylamine in their food (NCI 1979). The specific tissues examined were not reported and no studies of function were performed.

Hepatic Effects. 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 cells. Electron microscopy also revealed blebs, hypertrophy, and pleomorphism of the mitochondria. A NOAEL of 350 mg/kg/day was identified for hepatic effects, since no hepatic lesions were revealed by light microscopy.

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 2.2.2.2.

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.

Renal Effects. 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 g-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 frequemy 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.

Data from chronic-duration studies in rats and mice indicate that the bladder is a target organ for chronic oral exposure to *N*-nitrosodiphenylamine. 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 2.2.2.8).

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 2.2.2.1 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, lowdose, 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.

Dermal/Ocular Effects. Following chronic exposure to *N*-nitrosodiphenylamine, grossly observable cornea1 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 (l/20) (NCI 1979). While the authors 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.

Other Systemic Effects. 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/kgjday 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 does not appear to be related to treatment (NCI 1979). No histopathological lesions were observed in the salivary glands, pituitary, adrenals, or thyroid of rats and mice chronically exposed to *N*-nitrosodiphenylamine in their food (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.

2.2.2.3 immunological Effects

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

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.

2.2.2.4 Neurological Effects

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

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.

2.2.2.5 Developmental Effects

No studies were located regarding developmental effects in either humans or animals after oral exposure to *N*-nitrosodiphenylamine.

2.2.2.6 Reproductive Effects

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

No treatment-related histological effects of the testes, prostate, uterus, or ovaries 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.

2.2.2.7 Genotoxic Effects

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

Male mice given oral doses of *N*-nitrosodiphenylamine at 500 mg/kg showed no significant signs of testicular deoxyribonucleic acid (DNA) synthesis depression (Friedman and Staub 1976). Negative results were also obtained in a liver DNA fragmentation test in which male Sprague-Dawley rats were exposed to 540 mg/kg of *N*-nitrosodiphenylamine (Brambilla et al. 1987).

Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to *N*-nitrosodiphenylamine.

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 per 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 of Fischer-344 rats and $B6C3F_1$ mice (50/sex/strain) with the matched control groups consisting of 20 untreated rats and mice of each sex

(Cardy et al. 1979; NCI 197Y). 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 l979). 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 authors believe that the occurrence of these fibromas was 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. A carcinogenic potency factor for humans (q_1*) of 4.92×10^3(mg/kg/day)$^{-1}$ has been calculated by EPA (EPA 1980b) based on these findings. Using this q_1* estimated doses corresponding to individual lifetime upper-bound limits for increased risk of cancer have been calculated. These levels, 2×10^{-2} and 2×10^{-5} mg/kg/day, for increased risk in 1/10,000 and 1/100,000 people, respectively, are displayed graphically in Figure 2-l.

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 B6C3F$_1$ 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.

B6C3F$_1$and B6AKF$_1$ mice (l8sex/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 B6C3F $_1$ 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 B6C3F$_1$ mice in the later NCI (1979) study. This strain of mice may have a genetic tendency towards liver lesions.

IARC has concluded that no evaluation of the carcinogenicity of *N*-nitrosodiphenylamine to humans is currently possible, and there is limited evidence for the carcinogenicity of *N*-nitrosodiphenylamine in experimental animals (IARC 1982a; 1987).

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans or animals after dermal exposure to *N*-nitrosodiphenylamine.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, or renal effects in humans or animals after dermal exposure to *N*-nitrosodiphenylamine.

Dermal/Ocular Effects. No studies were located regarding dermal/ocular effects in humans after dermal exposure to *N*-nitrosodiphenylamine.

A single dermal study was located in which mice had 0.1 mL of a 0.1% solution of *N*-nitrosodiphenylamine painted on the intrascapular region once per week for 20 weeks (Iversen 1980). The author 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.

No studies were located regarding the following health effects in humans or animals after dermal exposure to *N*-nitrosodiphenylamine:

2.2.3.3 Immunological Effects
2.2.3.4 Neurological Effects
2.2.3.5 Developmental Effects
2.2.3.6 Reproductive Effects
2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located regarding cancer in humans after dermal exposure to *N*-nitrosodiphenylamine.

Single weekly 0.l-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.

2 . 3 TOXICOKINETICS

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

No studies were located regarding absorption of *N*-nitrosodiphenylamine in humans or animals following inhalation exposure.

2.3.1.2 Oral Exposure

Specific information on the rate and extent of absorption of *N*-nitrosodiphenylamine in humans or animals following oral exposure is not available. The appearance of metabolites in the urine of rats and in the serum of guinea pigs following oral administration provides indirect evidence of gastrointestinal absorption of *N*-nitrosodiphenylamine (Appel et al. 19%; Tatsumi et al. 1983). Furthermore, the occurrence of systemic effects in rats and mice in oral carcinogenicity studies suggests that Wnitrosodiphenylamine is absorbed through the gastrointestinal tract in these animals (Cardy et al. 1979; NCI 1979).

2.3.1.3 Dermal Exposure

No studies were located regarding absorption of *N*-nitrosodiphenylamine in humans after dermal exposure.

The appearance of lung adenomas in a dermal carcinogenicity study with mice provides indirect evidence of dermal absorption of *N*-nitrosodiphenylamine (Iversen 1980).

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution of *N*-nitrosodiphenylamine in humans or animals after inhalation exposure.

2.3.2.2 Oral Exposure

No studies were located regarding distribution of *N*-nitrosodiphenylamine in humans or animals after oral exposure.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution of *N*-nitrosodiphenylamine in humans or animals after dermal exposure.

2.3.3 Metabolism

2.3.3.1 Inhalation Exposure

No studies were located regarding metabolism of *N*-nitrosodiphenylamine in humans or animals after inhalation exposure.

2.3.3.2 Oral Exposure

No studies were located regarding metabolism of *N*-nitrosodiphenylamine in humans after oral exposure.

In experiments with animals, the reaction in which *N*-nitrosodiphenylamine is denitrosated to diphenylamine and nitric oxide seems to be the first step in the metabolic activation of *N*-nitrosodiphenylamine (Appel et al. 1984). A single dose of *N*-nitrosodiphenylamine in corn oil (1,000 mg/kg) was administered to female Wistar rats. Nitrate was identified as the major urinary metabolite, while nitrite, diphenylamine, and a monohydroxydiphenylamine were found in smaller amounts. The conclusion is that *N*-nitrosodiphenylamine is denitrosated to diphenylamine and nitric oxide. The nitric oxide is then converted into nitrite and nitrate. Nitrite is oxidized in substantial amounts to nitrate (Appel et al. 1984).

In vitro studies investigated the metabolism of *N*-nitrosodiphenylamine in phenobarbital-induced mouse liver microsomes (Appel et al. 1987a, 1987b, 1987c). The metabolites found were diphenylamine, 4-hydroxydiphenylamine, and its oxidized product, the corresponding quinoneimine. The authors conclude that diphenylamine undergoes ring hydroxylation to form 4-hydroxydiphenylamine which is oxidized to the quinoneimine. Since *N*-hydroxylation is recognized as the initial step in the bioactivation of carcinogenic arylamines, the *N*-hydroxy derivative of diphenylamine may be a potential metabolite. This possible metabolite, however, has not been detected using microsomal incubation. A postulated metabolic scheme based on these data is presented in Figure 2-2.

In vitro studies conducted with rat and mouse liver cytochrome *P*-450 demonstrated the denitrosation of *N*-nitrosodiphenylamine (Appel et al. 1979; Schrenk et al. 1982; Wakabayashi et al. 1982).

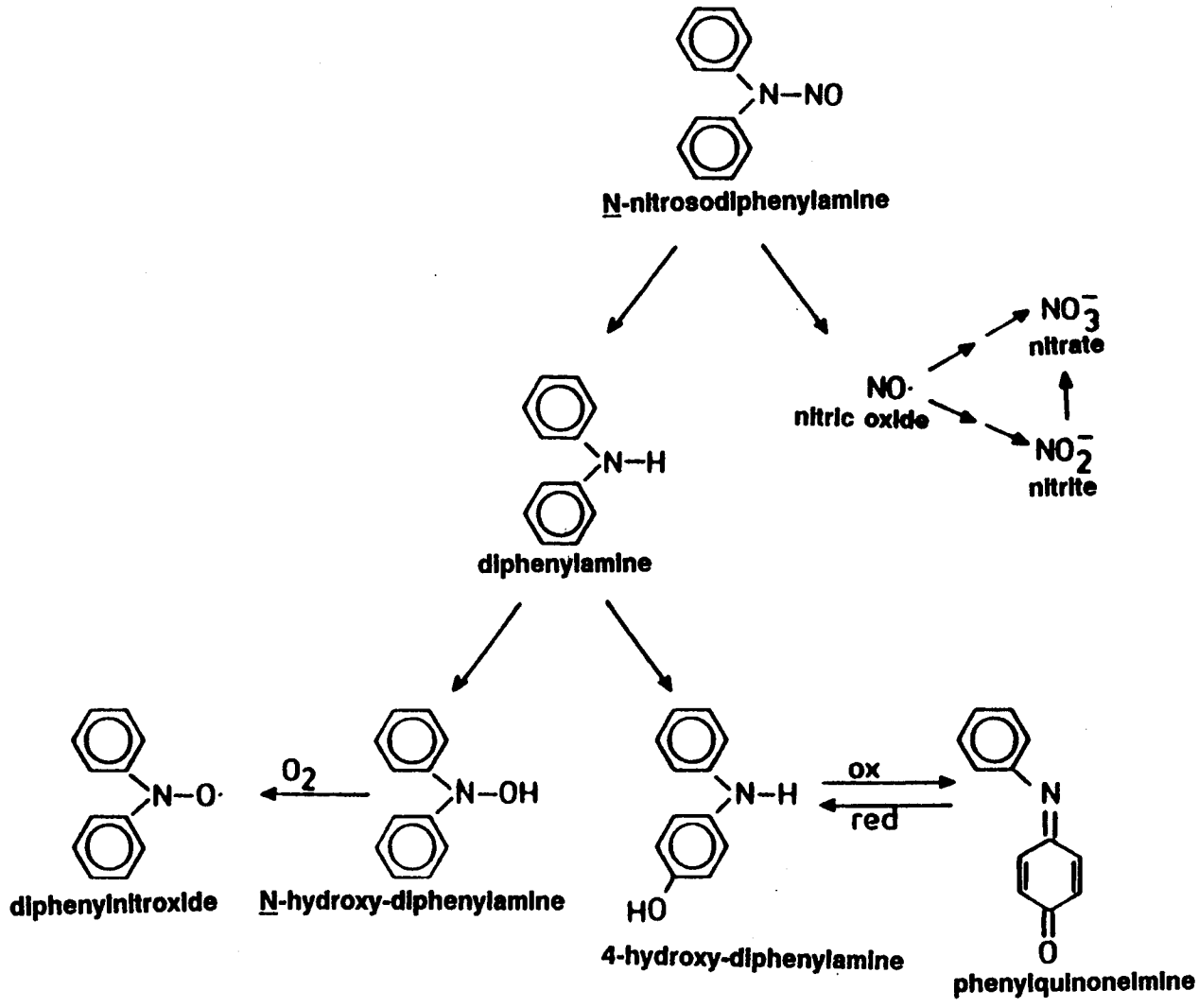
Transnitrosation of proline by .*N*-nitrosodiphenylamine occurred in male BD VI rats that were orally administered 28.28 mg/kg *N*-nitrosodiphenylamine and 50 μmol proline by gavage (Ohshima et al. 1982). The excretion of *N*-nitrosoproline was 15-fold higher than in the controls. Co-administration of thiocyanate had a catalytic effect, which resulted in a 58-fold increase in the urinary levels of *N*-nitrosoproline.

N-Nitrosodiphenylamine can undergo reductive metabolism by liver aldehyde oxidase under anaerobic conditions (Tatsumi et al. 1983). Guinea pigs received oral dosages (200 mg/kg) of *N*-nitrosodiphenylamine. Just before and 3 hours after administration of *N*-nitrosodiphenylamine, the guinea pigs were treated with oral dosages (50 mg/kg) of acetaldehyde (an electron donor). Acetaldehyde diphenylhydrazone was identified as a plasma metabolite.

2.3.3.3 Dermal Exposure

No studies were located regarding metabolism of *N*-nitrosodiphenylamine in humans or animals after dermal exposure.

FIGURE 2-2. Metabolic Pathways for N-Nitrosodiphenylamine*



*Adapted from Appel et al. 1987b

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

No studies were located regarding excretion of *N*-nitrosodiphenylamine in humans or animals after inhalation exposure.

2.3.4.2 Oral Exposure

No studies were located regarding excretion of iV-nitrosodiphenylamine in humans after oral exposure.

One study was located that investigated excretion in animals. After oral administration of a single l,000-mg/kg dose of *N*-nitrosodiphenylamine to female Wistar rats, the maximum urinary excretion of nitrate and nitrite was found 24-48 hours after administration (Appel et al. 1984). Within 36 hours of administration, 24.8% and 1.4% of the administered dose of *N*-nitrosodiphenylamine was excreted as nitrate and nitrite, respectively. Ninety-six hours after administration, about 30% of the administered dose had been eliminated as nitrite and nitrate.

2.3.4.3 Dermal Exposure

No studies were located regarding excretion of *N*-nitrosodiphenylamine in humans or animals after dermal exposure.

2.3.4.4 Other Routes of Exposure

In female Wistar rats, the maximum urinary nitrate or nitrite excretion was found in the 24 hours following intraperitoneal administration of 500 mg/kg *N*-nitrosodiphenylamine (Appel et al. 1984). This is a more rapid elimination than that following oral dosing. Ninety-six hours after administration, approximately 50% of the administered dose was detected as nitrate and nitrite--almost twice as much as was found after oral administration. Diphenylamine and hydroxydiphenylamine were also present as urinary metabolites. The rate of denitrosation after intraperitoneal injection was considerably higher than after oral administration. This was probably due to an altered availability of *N*-nitrosodiphenylamine to the liver.

Results from a study of rats, rabbits, and guinea pigs receiving 50 mg/kg *N*-nitrosodiphenylamine through intraperitoneal injection suggested that the rate of excretion of *N*-nitrosodiphenylamine into the bile and elimination of the chemical from the bile varies among species (Atawodi and Maduagwu 1990). Guinea pigs showed the most rapid excretion of *N*-nitrosodiphenylamine into the bile. Rabbits had the slowest excretion of *N*-nitrosodiphenylamine into the bile but the most rapid elimination of the chemical from the bile. Both excretion to and elimination from bile were comparatively slow in the rat. The half-lives for *N*-nitrosodiphenylamine elimination from bile for these species are as follows: 95 minutes for rabbits, 240 minutes for guinea pigs, and 510 minutes for rats.

2.4 RELEVANCE TO PUBLIC HEALTH

The general population is probably not exposed to *N*-nitrosodiphenylamine. *N*-Nitrosodiphenylamine is not a naturally occurring substance and is no longer manufactured in the United States. Although it has been shown to be produced by cultures of microorganisms under laboratory conditions, the extent to which this may occur in the environment is unknown. Available data indicate that it is not likely to be found

in air, water, or soil except in contaminated areas. The major routes of exposure to *N*-nitrosodiphenylamine for humans living near hazardous waste sites are probably via inhalation of airborne dust particles or ingestion of contaminated water. The result of direct skin contact with soil contaminated with *N*-nitrosodiphenylamine is unclear.

No information was located regarding toxic effects in humans following acute, intermediate, or chronic inhalation exposure to *N*-nitrosodiphenylamine; no occupational studies or case reports were located. Extremely limited animal data suggest that the primaty target of inhalation exposure to *N*-nitrosodiphenylamine is the respiratory system. There may also be some immunological and neurological effects.

No information was located regarding toxic effects in humans following acute, intermediate, or chronic oral exposure to *N*-nitrosodiphenylamine. Limited animal studies give data primarily on intermediate and chronic exposure. The target organ of *N*-nitrosodiphenylamine toxicity in rats, and possibly in mice, is the urinary bladder, although minor hepatic and ocular alterations have also been reported.

Chronic studies have reported that tumors occurred in rats and mice following a lifetime exposure to *N*-nitrosodiphenylamine in the diet. At lower doses, tumors were not evident. IARC has concluded that no evaluation of the carcinogenicity of *N*-nitrosodiphenylamine to humans is currently possible, and there is limited evidence for the carcinogenicity of *N*-nitrosodiphenylamine in experimental animals (IARC 1982a; 1587). Negative results have been found in in vivo tests and in vitro gene mutation and chromosome assays. However, there are conflicting results in in vitro human fibroblast DNA damage assays.

No information was located regarding toxic effects in humans following acute, intermediate, or chronic dermal exposure to *N*-nitrosodiphenylamine. Animal studies of poor quality found that *N*-nitrosodiphenylamine can irritate the skin.

No inhalation MRLs were derived because no human data and no reliable animal data exist. For the same reasons, no oral MRLs were derived for intermediate or acute exposure. No oral MRL was derived for chronic exposure because no human data exists and the only effects observed in a reliable animal study (epithelial hyperplasia and squamous metaplasia of the urinary bladder, seen in NCI 1979) were considered to be preneoplastic. No MRLs were derived for acute, intermediate, or chronic dermal exposure to Nnitrosodiphenylamine because appropriate MRL methodology has not been developed for this route.

Death. Although there have been no deaths reported in humans from *N*-nitrosodiphenylamine exposure, animal data suggest that fatalities can occur at high doses. Rats have been shown to be more susceptible to *N*-nitrosodiphenylamine toxicity than mice. Chronic studies by NCI (1979) found decreased survival in mice exposed to 711 mg/kg and in rats exposed to 200 mg/kg. An oral rat LD_{50} of 3,000 mg/kg was reported by Druckrey et al. (1967). There were no studies regarding lethality following inhalation exposure, which is assumed to be a likely route of exposure in humans.

Systemic Effects

Only limited histopathological data from an oral chronic-duration animal study are available for respiratory, cardiovascular, gastrointestinal, hematological, and musculoskeletal effects (NCI 1979). No pathological changes related to treatment were found in tissues from these systems that were analyzed. The significance of exposure in humans cannot be determined from these data.

Hepatic Effects. Evidence suggests that *N*-nitrosodiphenylamine may cause slight, but not major, damage to the liver at high oral doses in animals. An intermediate-duration study revealed pigmentation of Kupffer's cells in hepatic sinusoids in mice exposed to *N*-nitrosodiphenylamine in the diet. Electron microscopic examination revealed an increased number of smooth endoplasmic reticula distributed among glycogen granules, blebs, hypertrophy, and pleomorphism of the mitochondria in Swiss-Webster mice treated with *N*-nitrosodiphenylamine for 4 days (Nishie et al. 1972). These changes are believed to be representative of liver enzyme induction. Since there were no studies available to evaluate hepatotoxicity following inhalation exposure and there are no human data, judgments regarding the significance of these results for humans cannot be made.

Renal Effects. The urinary bladder is considered the target organ of *N*-nitrosodiphenylamine toxicity in animals. However, no data are available regarding bladder toxicity in humans. Data regarding the effects of acute exposure are not available, but chronic studies have reported toxic effects in the bladder of *N*-nitrosodiphenylamine-treated animals. Epithelial hyperplasia and a small degree of squamous metaplasia were evident in a few rats fed 50 and 200 mg/kg for 100 weeks (NCI 1979). There was a high incidence, of submucosal inflammation, as well as a minimal incidence of epithelial hyperplasia, of the urinary bladder in mice receiving 130-2,600 mg/kg *N*-nitrosodiphenylamine in the diet (NCI 1979). There is no information to indicate whether adverse bladder effects would occur in humans after exposure to *N*-nitrosodiphenylamine. However, other nitrosamines have been shown to adversely affect the bladder, and there is evidence to suggest that bladder cancer rates are higher in the rubber industry (the main industry using *N*-nitrosodiphenylamine) than in the general population (Boyland et al. 1968; NCI 1979). '

Dermal/Ocular Effects. A chronic oral study reported grossly observable cornea1 opacity in 15 of 50 male rats exposed to 200 mg/kg and 16 of 50 female rats exposed to 50 mg/kg (Cardy et al. 1979; NCI 1979). There is no information on whether exposure to this chemical may produce similar effects in humans. People living near hazardous waste sites could be at risk if the water supply were contaminated.

Other Systemic Effects. Decreases in body weight were observed in rats exposed to oral doses of *N*-nitrosodiphenylamine for intermediate and chronic durations, and in mice exposed chronically to the chemical (NCI 1979). The decrease was >l0% in rats exposed to levels of ≥200 mg/kg/day for 11 weeks, and the decrease was much more significant in females at 800 mg/kg/day (39% decrease relative to controls), the dose at which survival was also significantly decreased. The significance of body weight depression for humans cannot be determined from this study, and no additional data exist by which to assess the relevance to human health.

Immunological Effects. Limited histopathological data from a chronic animal study do not show adverse immunological effects due to oral exposure to *N*-nitrosodiphenylamine (NCI 1979). The relevance to human exposure cannot be determined from these data.

Neurological Effects. Limited histopathological data from a chronic animal study do not show adverse neurological effects due to oral exposure to *N*-nitrosodiphenylamine (NCI 1979). The relevance to human exposure cannot be determined from these data.

Developmental Effects. No studies were located regarding developmental effects in humans or animals after inhalation, oral, or dermal exposure to *N*-nitrosodiphenylamine. Since no information is available, the relevance to human exposure cannot be determined.

Reproductive Effects. Limited histopathological data from a chronic animal study do not show adverse reproductive effects due to oral exposure to *N*-nitrosodiphenylamine (NCI 1979). The relevance to human exposure cannot be determined from these data.

Genotoxic Effects. 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 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 2-2). 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 (see Table 2-3). A recessive lethal study involving Drosophila melanogaster produced a negative result as well (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 Drosophila 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 19Sl). 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.

Data from in vitro studies using prokaryotic and eukaryotic organisms and cultured mammalian cells are presented in Table 2-3. The response has been negative for the majority of gene mutation studies. However, two Salmonella assays detected gene mutations after exposure to metabolically activated *N*-nitrosodiphenylamine (Khudoley et al. 1987; Zielenska and Guttenplan 1988). *N*-Nitrosodiphenylamine exhibited no effect on mitotic crossing-over and gene conversion in Saccharomvces 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). Tests for DNA damage have produced mixed results among prokaryotes and fungi. Among mammalian hepatocytes, however, the results for DNA damage have been positive. As mentioned previously, only one study involving cultured human fibroblasts was positive for DNA damage (Snyder and Matheson 1985).

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 speculate 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

TABLE 2-2. Genotoxicity of *N*-Nitrosodiphenylamine In Vitro

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium (Ames assay)	Gene mutation	-	No data	Raineri et al. 1981
S. typhimurium (Ames assay)	Gene mutation	+	No data	Khudoley et al. 1987
S. typhimurium (liquid preincubation assay)	Gene mutation	+	No data	Zielenska and Guttenplan 1988
S. typhimurium (modified Ames assay)	Gene mutation	-	-	Probst et al. 1981
S. typhimurium (Ames assay)	Gene mutation	No data	-	Ichinotsubo et al. 1981
S. typhimurium (pour plate and preincubation assay)	Gene mutation	-	No data	Araki et al. 1984
S. typhimurium (Ames assay)	Gene mutation	-	-[a]	Crebelli et al. 1984
Escherichia coli (pour plate and preincubation assay)	Gene mutation	-	No data	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	-	No data	Shimada et al. 1989
E. coli (differential killing test)	DNA damage	+	No data	Green 1981
E. coli (differential killing test)	DNA damage	-	+	Tweats 1981
E. coli (rec assay)	DNA damage	-	No data	Mamber et al. 1983
E. coli (disc diffusion and liquid suspension assay)	DNA damage	-	-	Rosenkranz et al. 1981
Bacillus subtilis (rec assay)	DNA damage	-	+	Kada 1981

TABLE 2-2 (Continued)

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Eukaryotic organisms:				
Fungi:				
Schizosaccharomyces pombe (forward mutation assay)	Gene mutation	-	-	Loprieno 1981
Saccharomyces cerevisiae (rep-test)	DNA damage	No data	-	Kassinova et al. 1981
S. cerevisiae (rad assay)	DNA damage	-	+	Sharp and Parry 1981b
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	-	-	Kuroki et al. 1977
Chinese hamster (V79 cells)	Gene mutation	-	-	Jones and Huberman 1980
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
Rat (hepatocytes)	DNA damage	+	NA	Althaus et al. 1982
Rat (hepatocytes)	DNA damage	+	NA	Probst et al. 1981
Rat (hepatocytes)	DNA damage	+	NA	Sina et al. 1983
Rat (hepatocytes)	DNA damage	+	NA	Bradley et al. 1982
Rat (hepatocytes)	DNA damage	+	NA	Althaus and Pitot 1983

TABLE 2-2 (Continued)

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Mammalian cells (Cont.):				
Chinese hamster (hepatocytes)	DNA damage	+	NA	McQueen et al. 1983
Mouse (hepatocytes)	DNA damage	+	NA	McQueen et al. 1983
Human (fibroblasts)	DNA damage	+	No data	Snyder and Matheson 1985
Human (fibroblasts)	DNA damage	-	No data	Agrelo and Amos 1981
Human (fibroblasts)	DNA damage	-	No data	Martin and McDermid 1981
Chinese hamster (Don cells)	Chromsomal aberrations	No data	+/-	Abe and Sasaki 1977
Chinese hamster (fibroblasts)	Chromsomal aberrations	No data	+/-	Ishidate and Odashima 1977
Chinese hamster (ovary cells)	Sister chromatid exchange	-	-	Evans and Mitchell 1981
Chinese hamster (ovary cells)	Sister chromatid exchange	-	-	Perry and Thomson 1981
Chinese hamster (Don cells)	Sister chromatid exchange	No data	+	Abe and Sasaki 1977
Human (lymphocytes)	Sister chromatid exchange	(+)	No data	Lindahl-Kiessling et al. 1989

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

- = negative result; + = positive result; (+) = weakly or insignificantly positive; +/- = inconclusive; DNA = deoxyribonucleic acid; NA = not applicable

TABLE 2-3. Genotoxicity of *N*-Nitrosodiphenylamine In Vivo

Species (test system)	End point	Results	Reference
Nonmammalian cells:			
Drosophila melanogaster (recessive lethal test)	Gene mutation	-	Vogel et al. 1981
Host-mediated assays:			
Escherichia coli (D. melanogaster host-mediated assay)	DNA damager	+	Knasmuller et al. 1990
Mammalian cells:			
Rat (liver nuclei)	DNA damage	-	Brambilla et al. 1987
Mouse (bone marrow cells)	Micronuclei	-	McFee et al. 1989
Mouse (bone marrow cells)	Micronuclei	-	Salamone et al. 1981
Mouse (bone marrow cells)	Micronuclei	-	Tsuchimoto and Matter 1981
Mouse (testicular cells)	DNA synthesis inhibition	-	Friedman and Staub 1976
Mouse (cauda epididymis)	Abnormal spermal morphology	-	Topham 1981

- = negative result; DNA = deoxyribonucleic acid

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 (Crchelli cl al. 1984). Alternatively, transnitrosation could occur from *N*-nitrosodiphenylamine to another compound. Evidence exists for transnitrosation by *N*-nitrosodiphenylamine 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 activationj and the positive results obtained in the NCI (1979) dietary-carcinogenesis study in rats.

Cancer. The only neoplastic lesion shown to be significantly correlated with *N*-nitrosodiphenylamine csposure was an increase of bladder transitional cell carcinoma in rats (Cardy et al. 1979; NCI 1979). The difference was significant at only the higher of the two doses tested. Increases in other neoplastic lesions. including cancers of the integumentav system and liver, were found in orally exposed rats and mice (Cardy cl al. 1979); Innes et al. 1969; NCI 1968, 1979), but the increases were not statistically significant. Some early studies reported no treatment-related tumors in orally exposed rats (Argus and Hoch-Ligeti 1961; Druckrcy ct al. 1967); however, the bladder was not routinely examined in these studies. A nonsignificant increase in reticulum cell sarcomas was reported in 28-day-old mice subcutaneously injected with 1.000 mgikg *N*-nitrosodiphenvlamine and observed for 18 months (Innes et al. 1969; NCI 1968). EPA has calculated a q_1* of 4.92×10^{-3} $(mg/kg/day)^{-1}$ based on the bladder cancer in rats. According to EPA's Integrated Risk Information System (IRIS) database, *N*-nitrosodiphenylamine is a probable human carcinogen (class B2). According to IARC, *N*-nitrosodiphenylamine is a level 3 chemical, meaning there is not enough data to determine the potential carcinogenicity of this compound (IARC 1987). Given the equivocal nature of the data from most of these studies and the lack of information for humans, no statements regarding the possible carcinogenicity of *N*-nitrosodiphenylamine in humans can be made.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time biologic samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to N -nitrosodiphenylamine are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue

dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are often not substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by *N*-nitrosodiphenylamine are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "Populations That Are Unusually Susceptible."

2.5.1 Biomarkers Used to Identify or Quantify Exposure to *N*-Nitrosodiphenylamine

N-Nitrosodiphenylamine can be detected and quantitated in the blood, serum, and urine of animals, with the lowest detection limits for serum (Pylypiw and Harrington 1981). Limited animal data suggest that suspected metabolites of *N*-nitrosodiphenylamine can also be detected in the urine. However, these methods do not appear to have been used to test humans for exposure. and no monitoring data for *N*-nitrosodiphenylamine were located. Therefore, no conclusion regarding the usefulness of these potential hiomarkers in humans can be made, although it is reasonable to assume that they can indicate exposure. There are no other known biomarkers of exposure to *N*-nitrosodiphenylamine.

There are no data on how long *N*-nitrosodiphenylamine persists in the body of humans or animals. In one study, ninety-six hours after the administration of an oral dose, 30% of the dose had been eliminated in the urine (Appel et al. 1984). However, it is not known how much was eliminated in the feces or by other routes, and how much was retained in the body. No data are available regarding the exposure levels that would result in levels detectable in body fluids.

2.5.2 Biomarkers Used to Characterize Effects Caused by *N*-Nitrosodiphenylamine

Based on data in rats and mice, t,he target organ appears to be the urinary bladder. Observed effects consist of epithelial hyperplasia and squamous metaplasia of the bladder (NCI 1979). These effects were seen at the lowest dose tested (50 mg/kg/day), and the effect is only observable post-mortem. In addition, these effects can occur from other circumstances such as disease, exposure to drugs, and exposure to other chemicals, and are not unique to *N*-nitrosodiphenylamine. Therefore, they are not useful as specific hiomarkers of effect for *N*-nitrosodiphenylamine. There are so few data available on the chemical that it is difficult to associate specific symptoms with exposure to *N*-nitrosodiphenylamine.

2.6 INTERACTIONS WITH OTHER CHEMICALS

N-Nitrosodiphenylamine was mutagenic in strains TA98 and TA1535, but not TA100, in preincubation assays with rat liver S-Y fractions only in the presence of the comutagen norharman (9H-pyrido-[3,4b]indole) (Nagao and Takahashi 1981; Wakabayashi et al. 1981, 1982).

In mice treated with *N*-nitrosodiphenylamine prior to pentobarbital administration, pentobarbital sleeping time was significantly shortened compared to control mice given only the corn oil vehicle (Nishie et al. 1972). This was believed to be due to induction of liver enzymes that could metabolize pentobarbital.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceprible population will exhibit a different or enhanced response to *N*-nitrosodiphenylamine than will most persons exposed to the same level of *N*-nitrosodiphenylamine in the environment. Reasons include crenetic make-up, developmental stage, health and nutritional status. and chemical exposure history. These b parameters result in decreased function of the detoxification and excretory processes (mainly hepatic and renal) or the pre-existing compromised function of target organs. For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, "Populations With Potentially High Exposure."

It is difficult to determine persons with increased risk because there are limited data on the toxicity of *N*-nirrosodiphenylamine. People that have bladder dysfunction or disease may be more susceptible since rhe primary effect of *N*-nitrosodiphenylamine in animals is bladder cancer.

The induction of the hepatic microsomal enzymes, such as the mixed function oxidases, by *N*-nitrosodiphenylamine may affect the metabolism of some drugs and alcohol. The efficacy of prescription drugs may be altered because of the increased rate of metabolism.

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to *N*-nitrosodiphenylamine. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to *N*-nitrosodiphenylamine. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

Human exposure to *N*-nitrosodiphenylamine may occur by inhalation, ingestion, or dermal contact. The major routes of exposure to *N*-nitrosodiphenylamine for individuals living near hazardous waste sites are inhalation of airborne dust particles or ingestion of contaminated water. There is little actual experience in treatment of persons exposed to this compound. However, general recommendations for reducing absorption of *N*-nitrosodiphenylamine following acute exposure include removal from the source of exposure. In the case of inhalation exposure, the patient is moved to fresh air. If the eyes are exposed, they are irrigated with copious amounts of water. If dermal contact has occurred, contaminated clothing is removed and the exposed area is thoroughly washed with soap and water (HSDB 1992).

Following oral exposure, prevention of the absorption of *N*-nitrosodiphenylamine is imperative. The method used for reducing peak absorption is dependent on the amount ingested, the time since ingestion, and rhe patient's condition. Emesis may be considered unless the patient is comatose, is convulsing, or has lost rhe gag reflex. Caution concerning the use of this method stems from the risk of aspiration of vomit into the lungs. Gastric lavage may be used as an alternative to emesis. Endotracheal intubation may be performed to reduce the risk of aspiration pneumonia. Administration of a charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, has also been suggested (HSDB 1992).

2.8.2 Reducing Body Burden

There are no data regarding methods to enhance elimination of *N*-nitrosodiphenylamine. As in methods used with other chemicals, hemodialysis might be useful following acute intoxication, but no data were located regarding this possibility. After a single oral dose of *N*-nitrosodiphenylamine, the maximum urinary excretion of its metabolites (nitrate and nitrite) was found within 24-48 hours. The metabolites of Nnitrosodiphenylamine have been associated with hepatic and renal toxicity. No information was located on the hioaccumulation of *N*-nitrosodiphenylamine or its metabolites. However, there are data that indicate *N*-nitrosodiphenylamine has a low potential for bioaccumulation, based on the log K_{OW} (see Section 53.1).

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

Based on a multicompartmental pharmacokinetic model, Kadlubar et al. (1991) predicted that DNA adduct formation by 4-aminohiphenyl in the bladder epithelial cells would decrease with increasing frequency of voiding. A similar situation may exist with *N*-nitrosodiphenylamine. Since this compound causes bladder cancer in rats (Cardy et al. 1979; NCI 1979), increased voiding frequency might similarly mitigate its effects.

Like *N*-nitrosodiphenylamine, its metabolite diphenylamine has been associated with nephrotoxic effects. However, no information on the mechanism of action for nephrotoxicity of either chemical was located. Results from Wakabayashi et al. (1982) suggest that the carcinogenic effects of *N*-nitrosodiphenylamine may be due to interaction with other compounds. This study found that although *N*-nitrosodiphenylamine is not mutagenic in Salmonella tvphimurium TA98, it is mutagenic in the presence of norharman (β-carboline). Norharman has been found in a tobacco smoke, a tryptophan pyrolysate, and cooked foods. The effect was not due to transnitrosation, since a similar effect was seen with diphenylamine. However, other authors have suggested that transnitrosation of or by *N*-nitrosodiphenylamine could be mechanistically important. Since the mechanism of *N*-nitrosodiphenylamine carcinogenicity is unknown, no methods can be suggested for interfering with it.

Since there is no evidence that *N*-nitrosodiphenylamine bioaccumulates, the best mitigation would be to reduce absorption. If the carcinogenic metabolite were identified, it would be possible to consider interfering with its generation. However the metabolite is not known, and the metabolic enzymes involved have not been identified.

2.9 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, 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*-nitrosodiphenylamine is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of *N*-nitrosodiphenylamine.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce or eliminate the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of *N*-Nitrosodiphenylamine

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to *N*-nitrosodiphenylamine are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of *N*-nitrosodiphenylamine. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not he interpreted as "data needs" information (i.e., data gaps that must necessarily be filled).

No human data were located for *N*-nitrosodiphenylamine. The animal data on this chemical are limited. Most of the studies investigate the potential carcinogenicity of *N*-nitrosodiphenylamine following oral exposure: however, one dermal study provides information on the toxicity and carcinogenicity of the compound by this route. Oral data on toxicity come primarily from a carcinogenicity study in rats and mice conducted by the National Cancer Institute (NCI). Most of the data are based on extensive histological examination of tissues from exposed animals. although body weight and survival information were also provided. Several studies on the oral carcinogenicity of the chemical were located.

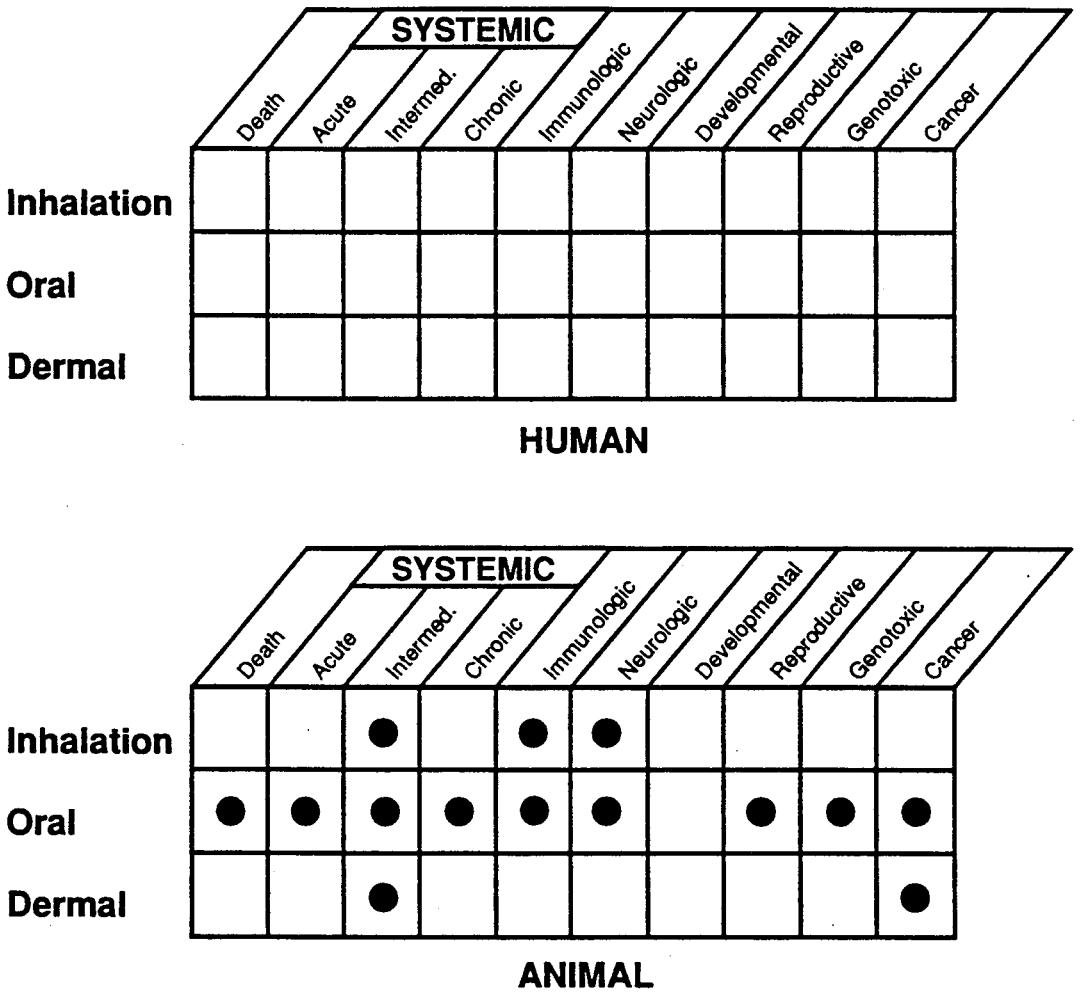
2.9.2 Identification of Data Needs

Acute-Duration Exposure. No cases of accidental or intentional poisonings were available to evaluate acute exposure in humans. There was a paucity of animal data, especially in animals exposed via inhalation or dcrmal routes. Enzyme induction in the liver was observed in mice receiving *N*-nitrosodiphenylamine by oral gavage for 4 days (Nishie et al. 1972). An acute oral LD_{50} was established for rats, but no inhalation LC_{50} or dermal LD_{50} studies were available (Druckrey et al. 1967). Insufficient information prevented the derivation of an acute-duration oral MRL. Pharmacokinetic data were not available to support the identification of target organs across routes of exposure. Inhalation and dermal data in mammalian species would be useful for determining possible effects of acute exposures in the population. The potential exists for the occurrence of acute exposure to *N*-nitrosodiphenylamine in populations near hazardous waste sites and accidental spills.

Intermediate-Duration Exposuye. There is no information on repeated exposure to *N*-nitrosodiphenylamine in humans. Rats showed body weight depression in an 8-11-week feeding study (NCI 1979). A low incidence of pigmentation of Kupffer's cells occurred in mice fed a diet containing a high concentration of *N*-nitrosodiphenylamine, but the effect was not considered adverse (NCI 1979). Well-conducted intermediate-duration inhalation and dermal studies would be useful in determining whether adverse effects occur via these exposure routes. Additional intermediate-duration oral studies that identify target organs and use several different animal species would be very helpful in determining potential adverse health effects in humans.

Chronic-Duration Exposure and Cancer. Chronic oral studies in rats have shown decreased body weight and bladder effects in the form of squamous metaplasia and submucosal inflammation (Cardy et al. 1979; NCI 1979). The only other noncancer health effect of *N*-nitrosodiphenylamine was cornea1 opacity in the high-dose male rats and low-dose female rats (Cardy et al. 1979; NCI 1979). These data indicate that the bladder is the target for chronic oral exposure to this chemical. A chronic oral MRL was not derived for *N*-nitrosodiphenylamine because the bladder effects were considered preneoplastic. Long-term animal studies via the inhalation and dermal routes would be valuable for determining whether similar chronic effects would occur, and if exposures via these routes could cause toxicity in populations exposed to *N*-nitrosodiphenylamine near hazardous waste sites for extended periods.

FIGURE 2-3. Existing Information on Health Effects of *N*-Nitrosodiphenylamine



● Existing Studies

No information was available on the carcinogenic potential of *N*-nitrosodiphenylamine in humans. Although contlicting cancer results have been seen in chronic bioassay there are enough oral exposure data to indicate that this chemical is carcinogenic in rats, primarily in the bladder. The only pertinent study of carcinogenicity following dermal exposure in mice lacked information that is critical for a thorough evaluation. The histological examinations were limited and no controls were used. Kinetic data suggest that *N*-nitrosodiphenylamine may be absorbed through the skin (Iversen 1980). More data concerning the actual risk of cancer from dermal exposure are needed since a possible route of exposure to humans is from contaminated soil.

Genotoxicity. Data from in vitro assays suggest that *N*-nitrosodiphenylamine and/or one or more of its metabolites may damage DNA in mammalian liver cells (McQueen et al. 1983). However, in vivo studies of this type are lacking. In addition, oral and perhaps even dermal exposure in vivo studies in animals would he useful since these are the routes of exposure pertinent to humans. Additional studies that investigate chromosomeichromatid effects in different animals and tissue/organ systems would help confirm or refute the inconclusive evidence (Abe and Sasaki 1977; Ishidate and Odashima 1977; McFee et al. 198'); Salamone et al. 1981) regarding this compound's clastogenicity. Genotoxicity assays in humans exposed to *N*-nitrosodiphenylamine would help to determine this chemical's status as a human genotoxin following in vivo exposure. Additional data on the metabolism of this compound would be very useful in assessing the inconsistencies of the available information.

Reproductive Toxicity. No human data and limited animal data were available regarding reproductive effects of *N*-nitrosodiphenylamine. Given the lack of reproductive information, any studies investigating adverse reproductive effects using different species and different routes of administration would be useful. Reproductive organ pathology could be examined in a 90-day study recommended under intermediateduration exposure.

Developmental Toxicity. There were no studies evaluating developmental effects in humans or animals. Data regarding potential developmental effects would be useful. Information is also lacking on the kinetics of *N*-nitrosodiphenylamine, such as its distribution and whether it is likely to cross the placenta.

Immunotoxicity. No studies were found that specifically investigated the immunotoxicity of *N*-nitrosodiphenylamine in either humans or animals. Studies specifically addressing the immune system responses in mammalian species would be valuable in assessing possible long-term health effects in humans that might retlect subtle changes in the immune system. Dermal studies may also provide useful information on the potential for allergic responses since skin contact by humans can occur in the workplace and via soil and water near hazardous waste sites.

Neurotoxicity. There were no human data and limited animal data evaluating the neurotoxicity of *N*-nitrosodiphenylamine. Given the lack of any information regarding neurotoxicity and the paucity of data concerning the mechanism of action of *N*-nitrosodiphenylamine, well-conducted acute, intermediate, and chronic studies across all exposure routes investigating neurological effects of *N*-nitrosodiphenylamine exposure would be useful.

Epidemiological and Human Dosimetry Studies. There are no epidemiological studies available on *N*-nitrosodiphenylamine. Populations that may potentially be exposed to *N*-nitrosodiphenylamine would include workers in the rubber industry, those residing near hazardous waste sites, or workers involved in the clea*N*-up of wastes. Rubber workers in cohort studies could be used as a potentially exposed population, although the generally low levels of the chemical that have been measured in the occupational

air space would make quantifying this relationship difficult. This type of epidemiological study may help determine whether bladder toxicity may occur in humans as in animals.

Biomarkers of Exposure and Effect. Currently. there are no hiomarkers identified for human exposure to *N*-nitrosodiphenylamine. The chemical and some of its metabolites have been measured in the blood. serum. and urine of animals (Pylypiw and Harrington 1981). Monitoring data in humans with suspected occupational exposure to *N*-nitrosodiphenylamine would be useful.

Currently. there are no human hiomarkers of effect identified for *N*-nitrosodiphenylamine. There are so few data available on the chemical that it is difficult to associate specific symptoms with exposure to *N*-nitrosodiphenylamine. The determination of the target organ in humans would be valuable for identifying possible effects to monitor in populations with high risk of exposure to the chemical, such as workers in the rubber industry. Furthermore. animal and epidemiological studies that correlate adverse health effects with levels in tissues would help researchers to devise more sensitive and more specific biomarkcrs of disease.

Absorption, Distribution, Metabolism, and Excretion. There was no information available on relative rates and extent of absorption, distribution, metabolism. and excretion for inhalation, oral, or dermal exposure in humans or animals. Although there are no quantitative data on absorption, animal studies gave indirect evidence that *N*-nitrosodiphenvlamine was absorbed following administration of a single oral &se (Appel et al. L1984; Tatsumi et al. 1983) and during chronic oral exposure (Cardy et al. 1979; NCI 1979). Absorption rate data for all three exposure routes would be useful in eslimating absorption characteristics in humans.

No studies on the distribution pattern and rates of *N*-nitrosodiphenylamine were available for humans or animals. Chronic oral studies have reported alterations in specific organs in animals (Cardy et al. 1979; NCI 1979); however, *N*-nitrosodiphcnylamine levels in these tissues were not provided. Additional studies on distribution would assist in the evaluation of target organ toxicity of *N*-nitrosodiphenylamine. Metabolism of *N*-nitrosodiphenylamine was studied in rats (Appel et al. 1984) and guinea pigs (Tatsumi et al. 1983) exposed to a single oral dose. No inhalation or dermal studies were available. Additional studies are needed to assess whether differences in rate and extent of metabolism exist across the three routes of exposure and to predict the metabolism pattern of the chemical in humans.

No human data and limited animal data were available on excretion. Rapid excretion occurs in rats after acute oral exposure (Appel et al. 1984). Studies on excretion following exposure via all routes would be useful for determining the variation in elimination pattern with route, and also the variation in excretion among species.

Comparative Toxicokinetics. No toxicokinetic information was available for humans. Pharmacokinetic data in animals, which could be used in the understanding of species differences in sensitivity and mechanism of toxicity to this chemical, are very limited (Appel et al. 1984; Atawodi and Maduagwu 1990; Ohshima ct al. 1982). Additional toxicokinetic studies in a variety of species would be useful indetermining the best animal model for evaluating *N*-nitrosodiphenylamine pharmacokinetic characteristics in humans. More toxicokinetic data would be helpful in assessing the potential for long-term health effects following chronic exposures, which are most likely to occur in residents living near hazardous waste sites.

Mitigation of Effects. Neither the mechanism of absorption of *N*-nitrosodiphenylamine, nor the mechanism of distribution in the body are known, although indirect evidence from animal studies indicates

that orally administered *N*-nitrosodiphenylamine is absorbed (Appel et al. 1984; Cardy et al. 1979; NCI 1979; Tatsumi et al. 1983). Information regarding these mechanisms would be useful in developing methods to reduce peak absorption. There are no established methods for reducing the body burden of this compound or any toxic metabolite(s), but the existing data suggest rhat *N*-nitrosodiphenylamine has a low potential for bioaccumulation (see Section 5.3.1). There is little actual experience in treating persons exposed to *N*-nitrosodiphenylamine. The mechanism of toxic action is not known, although possible carcinogenic mechanisms have been proposed (NCI 1979, Preussmann and Stewart 1984, Raineri et al. 1981; Wakabayashi et al. 1982). Information regarding the nephrotoxic and possible carcinogenic mechanisms of *N*-nitrosodiphenylamine would be useful in developing methods to block its toxic effects.

2.9.3 On-going Studies

No on-going studies were located for *N*-nitrosodiphenylamine.