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 1,2-diphenylhydrazine. 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 1,2-diphenylhydrazine, but may not be inclusive of the entire body of literature. A systematic review of the scientific evidence of the health effects associated with exposure to 1,2-diphenylhydrazine was also conducted; the results of this review are presented in Appendix C.

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 reliable inhalation or dermal data were identified for 1,2-diphenylhydrazine.

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 1,2-diphenyl-hydrazine 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 D). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

As illustrated in Figure 2-1, there are limited data on the toxicity of 1,2-diphenylhydrazine. No human studies were identified, and data from laboratory animal studies primarily come from a small number of oral studies. Nine studies published in three papers have examined the toxicity and carcinogenicity following oral exposure. Two additional oral studies only examined lethality and one study assessed carcinogenicity following dermal exposure. A chronic study in rats and mice was the only study examining a wide range of potential endpoints; other studies have focused on liver or body weight effects. No studies were located that evaluated possible effects on immunological, reproductive, or developmental function. Based on these data, the available studies suggest the following targets of toxicity:

- **Hepatic endpoint:** Hepatic effects are a presumed health effect for humans based on evidence from intermediate and chronic oral studies in rats and mice. Liver hypertrophy, bile duct duplication, and macrovesiculation was observed in rats after 13 weeks of dietary exposure; no alterations were observed after shorter exposure durations. After chronic exposure, fatty metamorphosis and coagulative necrosis were observed in rats and mice, respectively.

- **Cancer endpoint:** Increases in the incidences of neoplastic lesions in the liver, mammary gland, and Zymbal’s gland/ear canal/skin of ear were observed in chronically exposed rats. In mice, liver tumors were observed in females only.

- **Gastrointestinal endpoint:** Data are inadequate to conclude whether gastrointestinal effects will occur in humans. Inconsistent results have been observed in oral exposure animal studies. Intestinal hemorrhage was noted in mice exposed to 1,2-diphenylhydrazine in the diet for 4 weeks

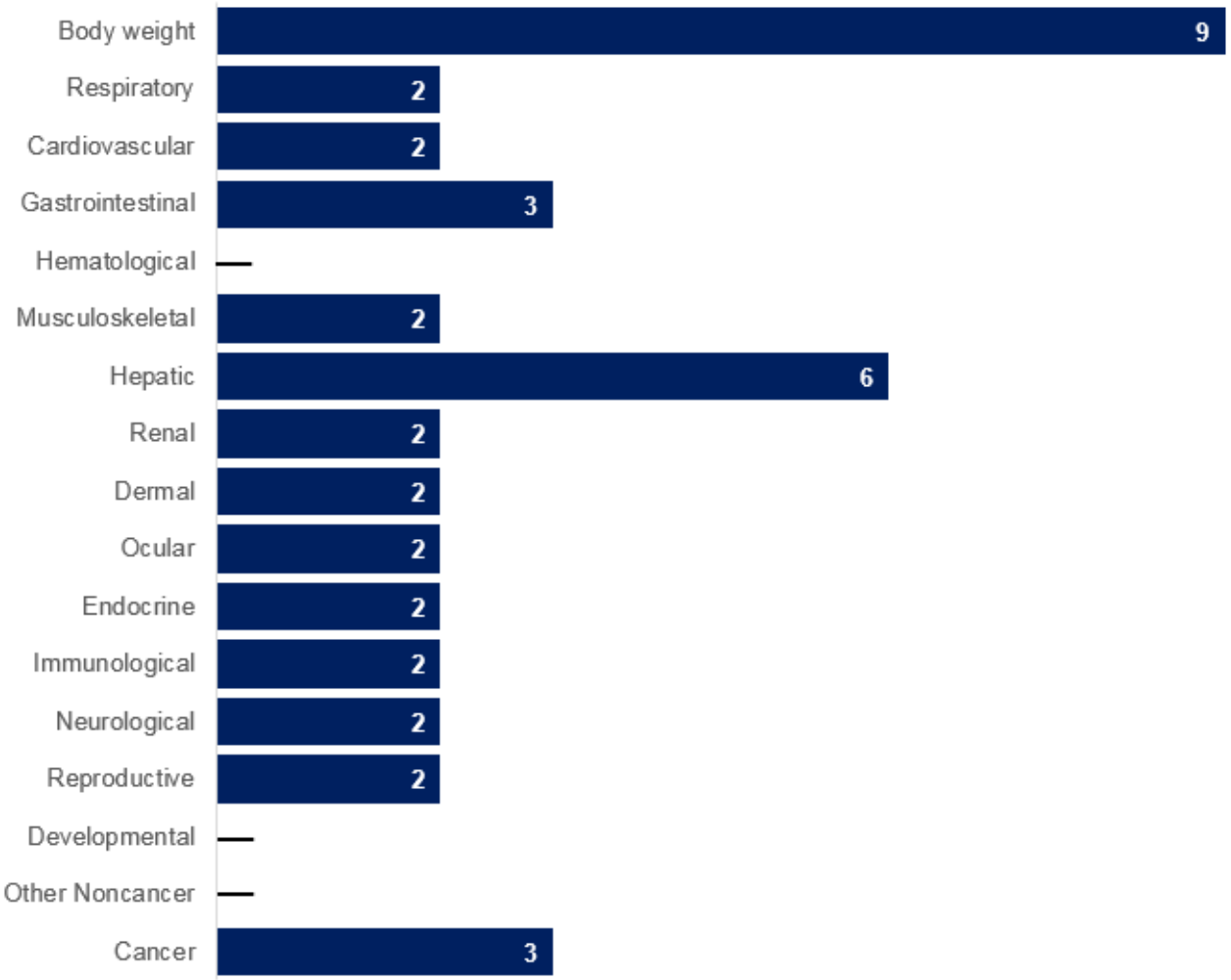
and stomach hyperkeratosis and/or acanthosis were observed in rats chronically exposed to 1,2-diphenylhydrazine in the diet.

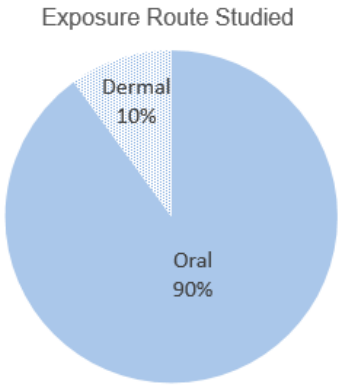
- **Respiratory endpoint:** Data are inadequate to conclude whether respiratory effects will occur in humans. Inconsistent results have been observed in oral exposure animal studies. Interstitial inflammation of the lungs was noted in rats chronically exposed to 1,2-diphenylhydrazine in the diet.

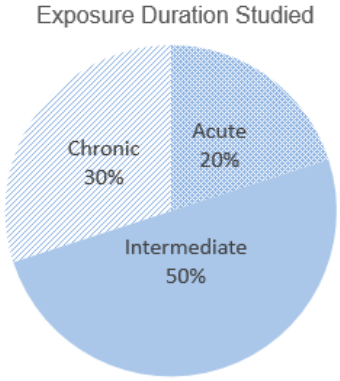
Figure 2-1. Overview of the Number of Studies Examining 1,2-Diphenylhydrazine Health Effects

Body weight, hepatic, gastrointestinal, and cancer effects of 1,2-diphenylhydrazine were the most widely examined potential toxicity outcomes

The majority of the studies examined oral exposure in **animals**; no data were identified for **humans** (counts represent studies examining endpoint)







*Includes studies discussed in Chapter 2. A total of 10 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to 1,2-Diphenylhydrazine – 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) 10 M	5 days (F)	0, 0.32, 1.26, 4.80, 10.3, 15.5	CS, BC, BW, HP, OW, FI, GN	Bd wt	15.5			
					Hepatic	15.5			Slight decrease (13%) in alkaline phosphatase at 15.5 mg/kg/day was not considered biologically relevant; no alterations in hepatic serum enzymes or liver histopathology
Dodd et al. 2012									
2	Rat (Fischer 344) 10 M	2 weeks (F)	0, 0.32, 1.26, 4.80, 10.3, 15.5	CS, BC, BW, HP, OW, FI, GN	Bd wt	15.5			
					Hepatic	15.5			Slight decrease (12%) in alkaline phosphatase at 15.5 mg/kg/day was not considered biologically relevant; no other alterations in hepatic serum enzymes or liver histopathology
Dodd et al. 2012									
3	Rat (Sprague-Dawley) 6–10 F	2 exposures 21 and 4 hours prior to sacrifice (G)	0, 60, 180	LE, BC, EA	Death				No increases in mortality
Kitchin et al. 1992									
4	Rat	Once (GW)	959	LE	Death			959	LD_{50}
Marhold et al. 1968									

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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
INTERMEDIATE EXPOSURE									
5	Rat (Fischer 344) 10 M	4 weeks (F)	0, 0.32, 1.26, 4.80, 10.3, 15.5	CS, BC, BW, HP, OW, FI, GN	Bd wt	15.5			
					Hepatic	15.5			13% and 26% reductions in serum alkaline phosphatase and aspartate aminotransferase, respectively, at 15.5 mg/kg/day were not considered biologically relevant; no alterations in liver histopathology
Dodd et al. 2012									
6	Rat (Fischer 344) 10 M	13 weeks (F)	0, 0.32, 1.26, 4.80, 10.3, 15.5	CS, BC, BW, HP, OW, FI, GN	Bd wt	15.5			
					Hepatic	4.80[b]	10.3		Slight to mild hypertrophy, minimal eosinophilic granular cytoplasm, minimal to slight multifocal bile duct duplication, and slight to mild multifocal macrovesiculation at ≥10.3 mg/kg/day. Reduction in serum alkaline phosphatase (19.7%) and aspartate aminotransferase (26%) at 15.5 mg/kg/day; no other alterations in hepatic serum enzymes
Dodd et al. 2012									
7	Rat	288 days (F)	0, 19	BW	Bd wt	19			
Marhold et al. 1968									

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8	Rat (Fischer 344) 5 M, 5 F	4 weeks (F)	M: 0, 3.5, 7, 14, 27, 54, 107, 150, 211 F: 0, 0.04, 0.15, 0.55, 1, 2, 7.5, 52, 365, 2,600	CS, BW, GN	Death			54 M 365 F	2/5 males died at 54 mg/kg/day; 100% mortality at higher doses
					Bd wt	211 M 2,600 F			
NCI 1978									
9	Mouse (B6C3F1) 5 M, 5 F	4 weeks (F)	M: 0, 9.1, 18, 36, 71, 140, 280, 391, 550; F: 0, 0.39, 1.04, 1.4, 2.6, 5.2, 19, 135, 950, 6,700	CS, BW, GN	Death			391 M 950 F	1/5 males and 4/5 females died
					Bd wt	550 M 6,700 F			
					Gastro			391 M 950 F	Intestinal hemorrhage
NCI 1978									
CHRONIC EXPOSURE									
10	Rat (Fischer 344 50 M, 50 F	78 weeks followed by 28–30-week recovery (F)	M: 0, 6.3, 24 F: 0, 3.7, 9.2	BW, GN, HP, CS	Death			9.2 F	Increased mortality
					Bd wt	6.3 M 3.7 F	24 M 9.2 F		Decreased body weight gain
					Resp		6.3 M		Interstitial inflammation of the lung in males at ≥6.3 mg/kg/day and females at 3.7 mg/kg/day but not at 9.2 mg/kg/day
					Cardio	9.2 F 24 M			
					Gastro	6.3 M	24 M 3.7 F		Hyperkeratosis and acanthosis of stomach in males at 24 mg/kg/day and acanthosis of the stomach in females at 3.7 mg/kg/day, but not at 9.2 mg/kg/day

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					Musc/skel	9.2 F 24 M			
					Hepatic	6.3 M 3.7 F	24 M 9.2 F		Fatty metamorphosis in females at 9.2 mg/kg/day and males at 24 mg/kg/day
					Renal	9.2 F 24 M			
					Dermal	9.2 F 24 M			
					Ocular	9.2 F 24 M			
					Endocr	9.2 F 24 M			
					Immuno	9.2 F 24 M			No histological alterations in immunological organs
					Neuro	9.2 F 24 M			No histological alterations in the brain
					Repro	9.2 F 24 M			No histological alterations in reproductive organs
					Cancer			6.3 M 9.2 F	CEL: hepatocellular carcinoma at ≥6.3 mg/kg/day in males only. Adrenal pheochromocytoma; squamous cell carcinoma in Zymbal's gland; and squamous cell carcinoma or papilloma in the ear canal, Zymbal's gland, and skin of the ear were observed in males at 24 mg/kg/day. In females, increases in liver neoplastic nodules and mammary gland adenocarcinomas were observed at 9.2 mg/kg/day.

NCI 1978

Table 2-1. Levels of Significant Exposure to 1,2-Diphenylhydrazine – 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
11	Mouse (B6C3F1) 50 M, 50 F	78 weeks followed by 28–30-week recovery (F)	M: 0, 14, 69 F: 0, 6.9, 69	LE, HP	Death			69	Increased mortality
					Bd wt	14 M 6.9 F		69 M,F	Decreased body weight gain (36%)
					Resp	69			
					Cardio	69			
					Gastro	69			
					Musc/skel	69			
					Hepatic	6.9 F	69 F		Coagulative necrosis
					Renal	69			
					Dermal	69			
					Ocular	69			
					Endocr	69			
					Immuno	69			No histological alterations in immunological organs
					Neuro	69			No histological alterations in the brain
					Repro	69			No histological alterations in reproductive organs
					Cancer			69 F	CEL: hepatocellular carcinoma in females

NCI 1978

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[b]Used to derive an intermediate-duration oral MRL of 0.05 mg/kg/day based on a NOAEL of 4.80 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability)

BC = biochemistry; Bd wt or BW = body weight; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; (G) = gavage; Gastro = gastrointestinal; GN = gross necropsy; HP = histopathology; immuno = immunological; LD_{50} = lethal dose, 50% mortality; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; OW = organ weight; Repro = reproductive; Resp = respiratory

Highlighted rows indicate MRL principal study.

Figure 2-2. Levels of Significant Exposure to 1,2-Diphenylhydrazine – Oral
Acute (≤14 days)

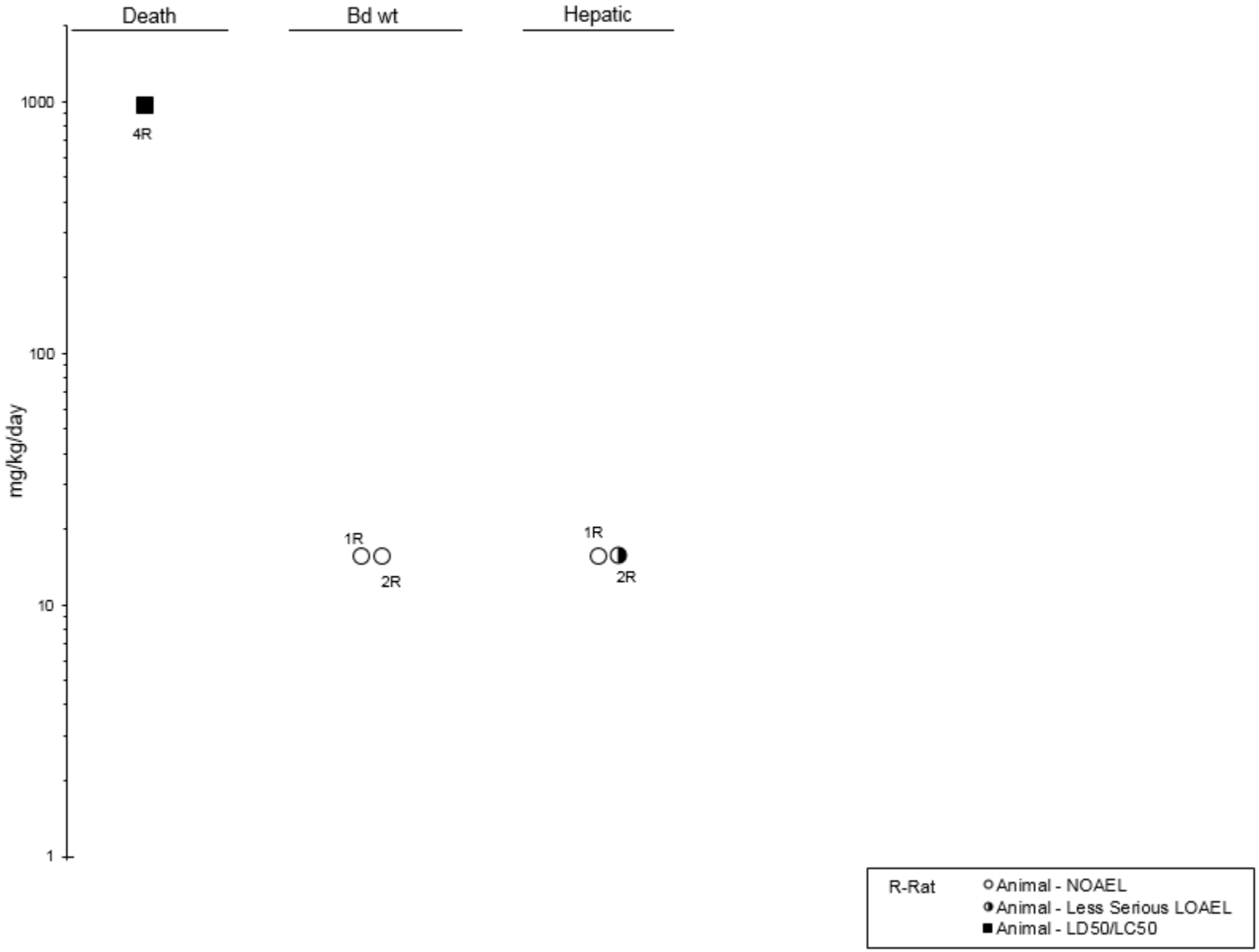


Figure 2-2. Levels of Significant Exposure to 1,2-Diphenylhydrazine – Oral
Intermediate (15-364 days)

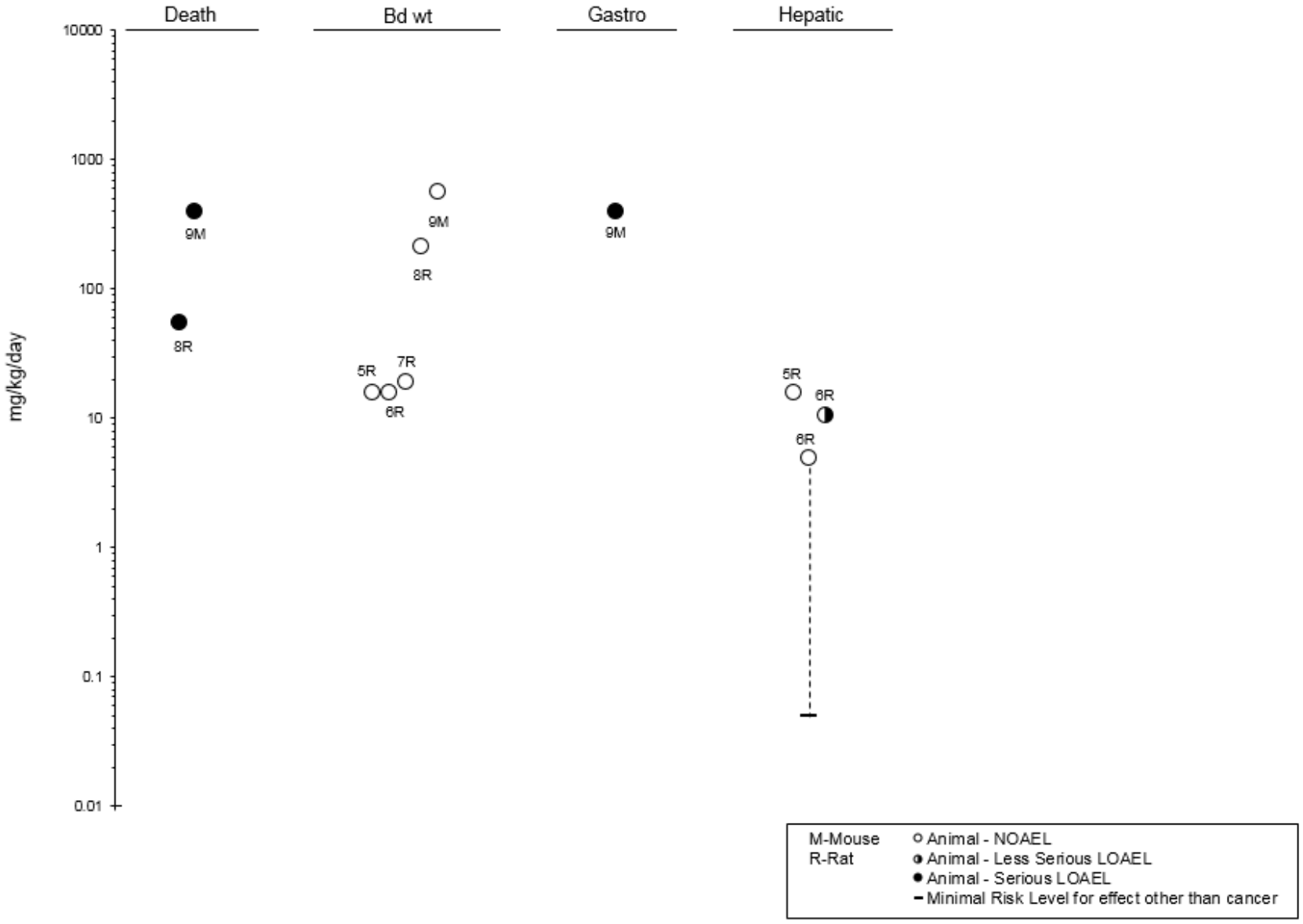


Figure 2-2. Levels of Significant Exposure to 1,2-Diphenylhydrazine – Oral

Chronic (≥365 days)

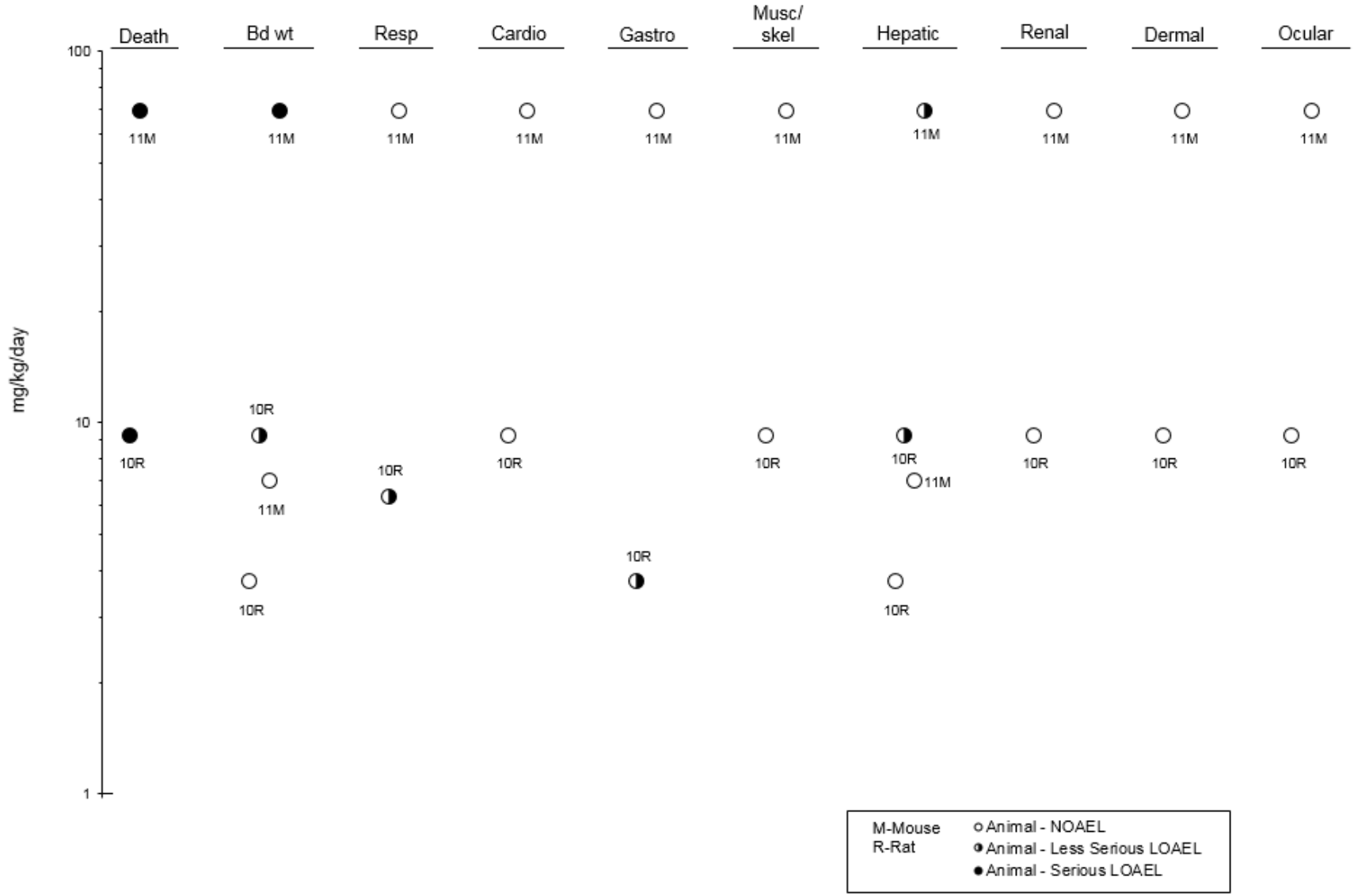
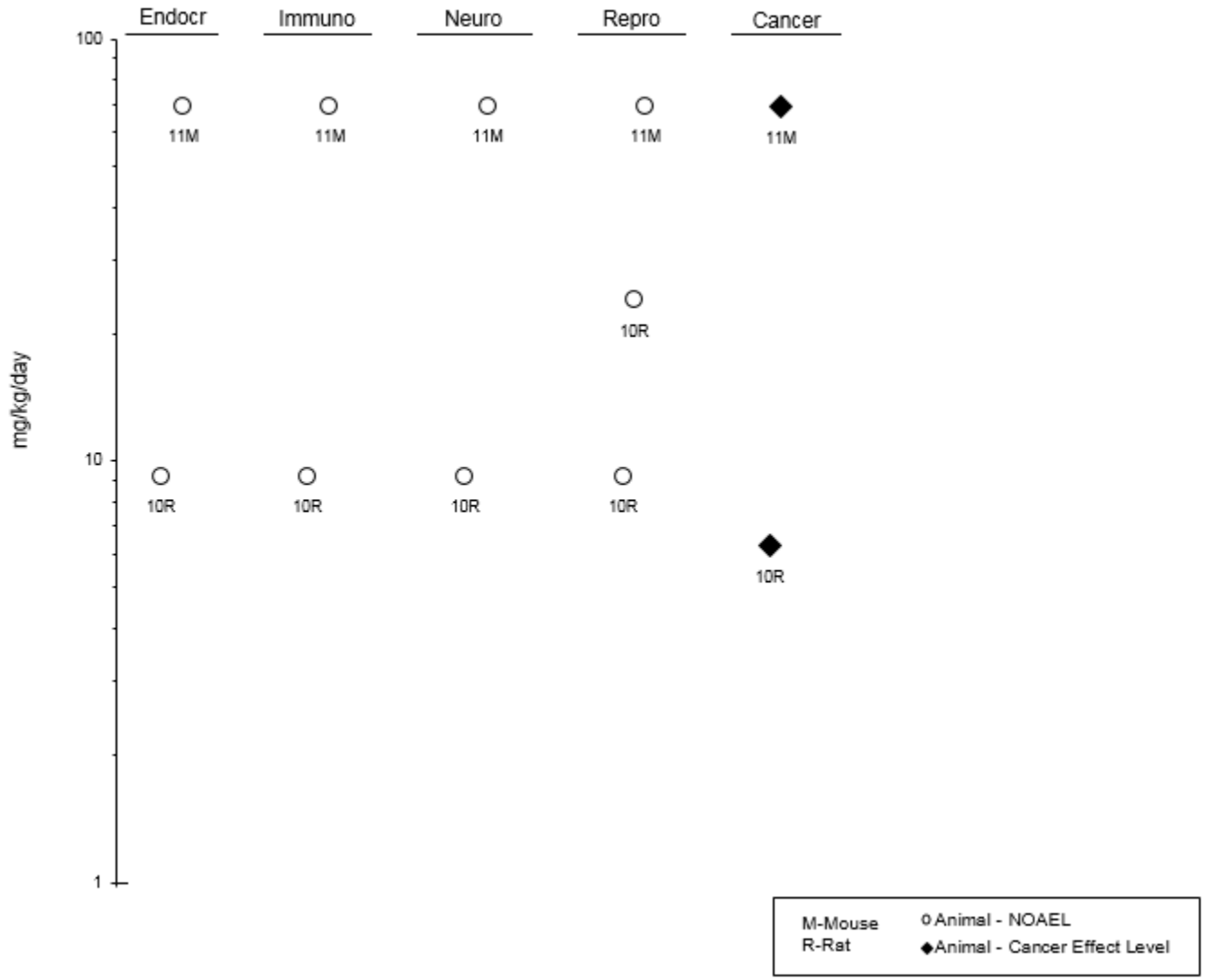


Figure 2-2. Levels of Significant Exposure to 1,2-Diphenylhydrazine – Oral
Chronic (≥365 days)



2.2 DEATH

Limited information is available regarding the lethality of orally administered 1,2-diphenylhydrazine in animals. An incompletely documented acute LD_{50} of 959 mg/kg in rats (Marhold et al. 1968) and an unreliable acute lethal dose 1,213 mg/kg/day in wild deer mice (Schafer and Bowles 1985) have been reported. Interpretation of the results of the Schafer and Bowles (1985) study is limited by the method used to measure dose (estimated from the number of 1,2-diphenylhydrazine-treated seeds consumed) and the lack of information on the actual number of deaths was not reported. No deaths were observed in rats administered two gavage doses of 180 mg/kg (sacrificed within 21 hours of last dose) (Kitchin et al. 1992) or rats exposed to 15.5 mg/kg/day in the diet for 5 days or 2 weeks (Dodd et al. 2012).

In repeated exposure studies, deaths were observed in rats and mice exposed to 54 or 390 mg/kg/day, respectively, for 4 weeks (NCI 1978). In another study, no deaths were observed in rats fed up to 15.5 mg/kg/day 1,2-diphenylhydrazine for 4 weeks or 13 weeks (Dodd et al. 2012). In a chronic dietary study, increases in mortality were observed in female rats exposed to 9.2 mg/kg/day and male and female mice exposed to 69 mg/kg/day (NCI 1978).

2.3 BODY WEIGHT

Chronic (NCI 1978), but not acute (Dodd et al. 2012) or intermediate (Dodd et al. 2012; Marhold et al. 1968; NCI 1978) oral exposure to 1,2-diphenylhydrazine led to significant alterations in body weight in laboratory animals. Male rats treated with 1,2-diphenylhydrazine in the diet at a dose of 24 mg/kg/day for 78 weeks had approximately l0–15% decreased body weight gain (NCI 1978); food consumption data were not reported. Decreased weight gain (approximately 36% at termination of the study) was observed in male and female mice exposed to 69 mg/kg/day in the diet for 78 weeks (NCI 1978).

2.4 RESPIRATORY

Respiratory effects occurred in rats after chronic exposure to 1,2-diphenylhydrazine in the diet for 78 weeks (NCI 1978); the incidences of interstitial inflammation of the lungs were significantly increased in male rats exposed to 6.3 or 24 mg/kg/day and in females at 3.7 mg/kg/day, but not at 9.2 mg/kg/day (NCI 1978).

2.5 CARDIOVASCULAR

No histological alterations were observed in rats or mice chronically exposed to doses as high as 24/9.2 (males/females) or 69 mg/kg/day, respectively (NCI 1978).

2.6 GASTROINTESTINAL

Intestinal hemorrhages were noted in mice exposed to lethal doses (≥390 mg/kg/day) for 4 weeks (NCI 1978). The severity and incidences of the hemorrhage were not described. Statistically increased incidences of hyperkeratosis and acanthosis in the stomach occurred in male rats at 24 mg/kg/day and acanthosis was observed in female rats at 3.7 mg/kg/day 1,2-diphenylhydrazine in the diet for 78 weeks (NCI 1978); the incidence in female rats administered 9.2 mg/kg/day (11%) was not significantly different from concurrent controls (4%). No gastrointestinal lesions were observed in mice treated with doses up to 69 mg/kg/day (NCI 1978).

2.7 HEMATOLOGICAL

No studies were located that evaluate hematological effects in animals following exposure to 1,2-diphenylhydrazine by inhalation, oral, or dermal routes. In a single study, intravenous injection of an 18.4 mg/kg dose of 1,2-diphenylhydrazine did not cause methemoglobinemia in rats, although methemoglobin was formed by an equimolar dose of aniline (Pfordte 1973).

2.8 MUSCULOSKELETAL

No histopathological alterations were observed in the musculoskeletal system of rats or mice exposed to 9.2/24 or 69 mg/kg/day, respectively, in the diet for 78 weeks (NCI 1978).

2.9 HEPATIC

Some hepatic alterations have been observed in male rats exposed to ≤15.5 mg/kg/day 1,2-diphenylhydrazine in the diet for 5 days or 2 weeks (Dodd et al. 2012). The alterations consisted of <10% statistically significant increases in relative liver weight at 15.5 mg/kg/day for 5 days or ≥4.80 mg/kg/day for 2 weeks and decreases in serum alkaline phosphatase levels (<15%) at 15.5 mg/kg/day for 5 days or 10.3 mg/kg/day for 2 weeks. There were no changes in serum alanine aminotransferase, aspartate aminotransferase, total bilirubin, or lactate dehydrogenase levels, and no histopathological changes were observed following acute exposure. In the absence of histological

alterations, the slight changes in relative liver weight and serum alkaline phosphatase levels were not considered toxicologically relevant. Rats treated by gavage at 21 and 4 hours prior to sacrifice with 60 or 180 mg/kg had no alterations in alanine aminotransferase (Kitchin et al. 1992).

Exposure via the diet to 10.3 or 15.5 mg/kg/day for 13 weeks resulted in increases in the incidences of slight/mild hypertrophy, eosinophilic granular cytoplasm, and multifocal bile duct duplication; multifocal macrovesiculation was also observed at 15.5 mg/kg/day (Dodd et al. 2012). However, no histological alterations were observed in rats exposed to up to 15.5 mg/kg/day for 4 weeks (Dodd et al. 2012). Decreases in alkaline phosphatase (7–13%) and aspartate aminotransferase (17–26%) were also noted at 15.5 mg/kg/day in rats exposed for 4 or 13 weeks.

Chronic exposure resulted in histological alterations in rats and mice exposed to 1,2-diphenylhydrazine in the diet for 78 weeks (NCI 1978). In rats, the lesions included increased fatty metamorphosis of the liver in male and female rats at 24 and 9.2 mg/kg/day, respectively. However, the increased incidence in 9.2 mg/kg/day female rats was only statistically significantly when compared to the low-dose control group due to the high incidence observed in the high-dose control group (12% in the high-dose controls compared to 4% in the low-dose controls). Coagulative necrosis was observed in female mice at 69 mg/kg/day, but was not observed in male mice. Other liver alterations were noted in the NCI (1978) chronic rat and mouse study, but the incidences were not dose-related.

Current hypotheses relating to the hepatic effects of 1,2-diphenylhydrazine exposure in animals include possible contributions of cytochrome P450 induction to the development of hepatic hypertrophy; the involvement of peroxisome proliferation in developing eosinophilic granular cytoplasm; aberrant lipid metabolism or transport contributing to hepatocyte cytoplasm macrovesiculation; and epithelial cell injury or hepatic necrosis that could have induced biliary duct duplication (Dodd et al. 2012).

2.10 RENAL

No significant histological alterations in the kidney were observed in rats or mice chronically treated for 78 weeks with up to 24/9.2 (males/females) or 69 mg/kg/day, respectively (NCI 1978).

2.11 DERMAL

No significant histological alterations in the skin were observed in rats or mice chronically exposed to 24/9.2 or 69 mg/kg/day, respectively (NCI 1978).

2.12 OCULAR

No significant histological alterations in ocular tissues were observed in rats exposed to 24/9.2 mg/kg/day or mice exposed to 69 mg/kg/day 1,2-diphenylhydrazine in the diet for 78 weeks (NCI 1978).

2.13 ENDOCRINE

No non-cancerous histological alterations were observed in the adrenal or thyroid glands of rats or mice chronically exposed to doses as high as 24/9.2 or 69 mg/kg/day 1,2-diphenylhydrazine, respectively, in the diet for 78 weeks (NCI 1978).

2.13 IMMUNOLOGICAL

No studies examined immune function following exposure to 1,2-diphenylhydrazine. Chronic exposure in the diet of rats or mice to 24/9.2 or 69 mg/kg/day, respectively, did not result in histological alterations in the bone marrow, spleen, or lymph nodes (NCI 1978).

2.15 NEUROLOGICAL

Rats and mice chronically treated with 1,2-diphenylhydrazine in the diet did not show symptoms of toxicity or histological alterations in the brain (NCI 1978), but no behavioral or neurological evaluations were conducted.

2.16 REPRODUCTIVE

Reproductive function has not been evaluated in laboratory animals. The NCI (1978) chronic study of rats exposed to 24 mg/kg/day (males) or 9.2 mg/kg/day (females) and of mice exposed to 69 mg/kg/day (males and females) did not find histological alterations in the reproductive tissues.

2.17 DEVELOPMENTAL

No studies were located regarding developmental effects of 1,2-diphenylhydrazine in humans or animals by any route of exposure.

2.18 OTHER NONCANCER

No studies examining other noncancer effects were identified.

2.19 CANCER

The carcinogenicity of 1,2-diphenylhydrazine has been investigated in oral, dermal, and parenteral studies in laboratory animals. Treatment-related neoplasms occurred in rats and mice that were treated with low or high doses of 1,2-diphenylhydrazine in the diet for 78 weeks, followed by untreated observation periods of 28 or 30 weeks (rats) and 17 or 18 weeks (mice) (NCI 1978); tumor incidences were calculated as combined incidences for animals dying early, sacrificed at 78 weeks, or at the end of the observation period. Male rats had statistically significant increased incidences of hepatocellular carcinomas and/or neoplastic nodules in the liver at 6.3 and 24 mg/kg/day. At 24 mg/kg/day, there were squamous-cell carcinomas of the Zymbal's gland and squamous cell carcinomas or papillomas of the ear canal, Zymbal's gland, and skin of the ear (combined incidences). The incidence of adrenal pheochromocytomas or malignant pheochromocytomas was significantly higher in the 24 mg/kg/day male rats ($p=0.042$ for the Fisher exact test), as compared to controls; however, the result was not significant under the Bonferroni criteria. Incidences of liver neoplastic nodules and mammary gland adenocarcinomas were increased significantly in female rats treated with 6.3 mg/kg/day, but not 3.7 mg/kg/day. A significantly increased incidence of hepatocellular carcinoma occurred in female mice treated with 69 mg/kg/day, but not 6.9 mg/kg/day. Doses of 14 or 69 mg/kg/day were not neoplastic for male mice. ATSDR notes that the nomenclature for classifying proliferative hepatocellular lesions was revised and the term "neoplastic nodule" is no longer recommended by the National Toxicology Program (NTP) to describe lesions that would now be termed hepatocellular hyperplasia or hepatocellular adenoma (Maronpot et al. 1986a).

In other studies, tumors were not observed in male rats treated with 19 mg/kg/day doses of 1,2-diphenyl-hydrazine in the diet for life (mean survival time=288 days) (Marhold et al. 1968). The significance of this finding is uncertain because the type and scope of pathological examination were not reported. Pliss (1974) reported increased numbers of tumors of the liver, Zymbal's gland, mammary gland, and other sites in rats that were treated with 1,2-diphenylhydrazine in the diet at an estimated dose of 85 mg/kg/day, 5 days/week for 588 days (Pliss 1974). These findings are inconclusive, however, because of lack of control data and other report inadequacies.

Inconclusive data for carcinogenicity of dermally applied 1,2-diphenylhydrazine in mice are available. Dermal application of an estimated 1,2-diphenylhydrazine dose of 63 mg/kg/day 3 times/week for

442 days caused a 22.2% incidence of tumors in mice (Pliss 1974). Tumors occurred in the lung, liver, and other tissues, and the tumor incidence in control mice was 17%. The significance of these findings cannot be determined, as incidences of specific tumors in the control group were not reported.

Intraperitoneal administration of 200 mg/kg 1,2-diphenylhydrazine 3 times/week for 8 weeks resulted in increases in the incidence of lung tumors in male mice; evidence in female mice was considered equivocal (Maronpot et al. 1986b). Increases in tumors have also been observed in other studies involving subcutaneous injection in rats and mice (Genin et al. 1975; Kurliandskiĭ et al. 1976; Pliss 1974; Shabad and Genin 1975; Spitz et al. 1950); however, the results are inconclusive due to inadequate reporting and other limitations.

Based on sufficient evidence of carcinogenicity in laboratory animal studies, the Department of Health and Human Services concluded that 1,2-diphenylhydrazine is reasonably anticipated to be a human carcinogen (NTP 2016) and EPA concluded that it is a probable human carcinogen (Group B2) (IRIS 2006).

2.20 GENOTOXICITY

The genotoxicity of 1,2-diphenylhydrazine has been evaluated in a limited number of *in vitro* and *in vivo* studies. No studies were located regarding the genotoxicity of 1,2-diphenylhydrazine in humans by any route of exposure. A limited number of assays have been conducted using bacteria or mammalian cells. As indicated in Table 2-2, 1,2-diphenylhydrazine was mutagenic in *Salmonella typhimurium* (Dunkel et al. 1985; Haworth et al. 1983), but not in *Escherichia coli* (Dunkel et al. 1985). Exogenous metabolic activation systems were necessary for expression of the aforementioned effects. In mammalian cell culture, 1,2-diphenylhydrazine produced chromosome aberrations and sister chromatid exchanges in Chinese hamster cells (Galloway et al. 1987). Ohnishi et al. (2000) reported deoxyribonucleic acid (DNA) damage in calf thymus DNA fragments incubated with a 10% (v/v) ethanol solution of 1,2-diphenylhydrazine. The addition of 20 µM copper(II) chloride increased the DNA damage.

Table 2-2. Genotoxicity of 1,2-Diphenylhydrazine *In Vitro*

Species (test system)	Endpoint	Results: Activation With	Results: Activation Without	Reference
Prokaryotic organisms				
Salmonella typhimurium (plate incorporation)	Gene mutation	(+)	–	Dunkel et al. 1985
S. typhimurium (plate incorporation)	Gene mutation	+	–	Haworth et al. 1983
Escherichia coli WP2uvrA	Gene mutation	–	–	Dunkel et al. 1985
Mammalian cells				
Chinese hamster ovary cells	Chromosome aberrations	+	+/–	Galloway et al. 1987
Chinese hamster ovary cells	Sister chromatid exchange	+	–	Galloway et al. 1987

+ = positive results; (+) = weakly positive results; +/– = inconclusive; – = negative results

In *in vivo* studies (Table 2-3), 1,2-diphenylhydrazine inhibited testicular DNA synthesis in mice when administered as a single 100 mg/kg intraperitoneal injection (Seiler et al. 1977), but did not cause hepatic DNA damage in rats administered two oral doses of 180 mg/kg, at 21 and 4 hours before sacrifice (Kitchin et al. 1994). Exposure by feed or injection did not cause sex-linked recessive lethal mutations in *Drosophila* (Yoon et al. 1985).

Table 2-3. Genotoxicity of 1,2-Diphenylhydrazine *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Invertebrate systems			
Drosophila melanogaster (feeding)	Sex-linked recessive lethal mutation	–	Yoon et al. 1985
D. melanogaster (injection)	Sex-linked recessive lethal mutation	–	Yoon et al. 1985
Laboratory animal evidence			
Mouse (strain not reported) (intraperitoneal injection)	DNA damage; inhibition of testicular DNA synthesis.	+	Seiler et al. 1977
Sprague-Dawley rat (gavage)	DNA damage (hepatic DNA alkaline elution)	–	Kitchin et al. 1994

– = negative result; + = positive result