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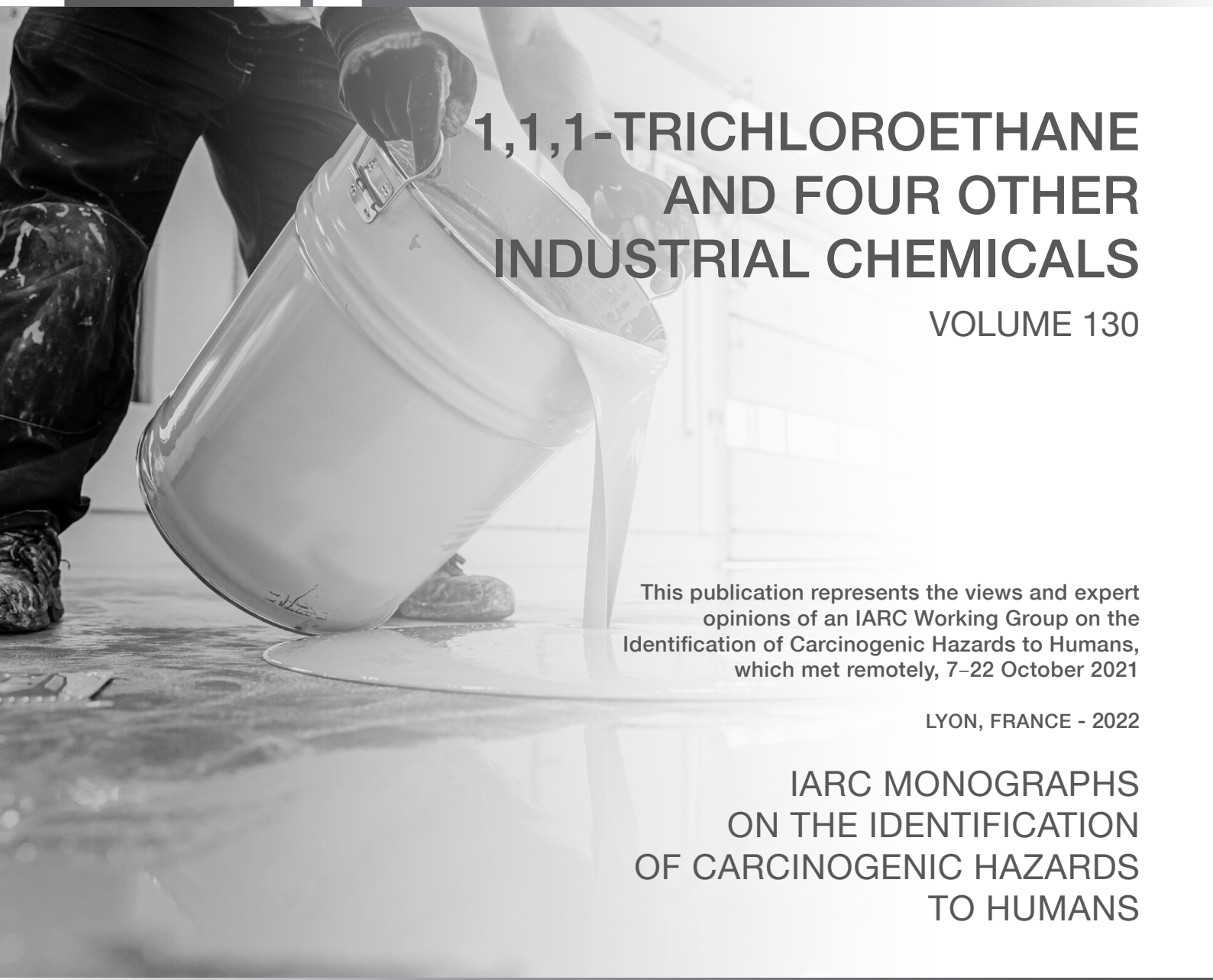

1,1,1-TRICHLOROETHANE AND FOUR OTHER INDUSTRIAL CHEMICALS

VOLUME 130

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International Agency for Research on Cancer

World Health Organization

1,2-DIPHENYLHYDRAZINE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 122-66-7

EC/List No.: 204-563-5

Chem. Abstr. Serv. name: 1,2-diphenylhydrazine

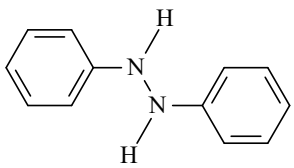
IUPAC systematic name: 1,2-diphenylhydrazine

Synonyms: hydrazobenzene; *N*,*N*′-diphenylhydrazine; hydrazodibenzene; *N*,*N*′-bianiline; 1,2-diphenyldiazane; symmetrical diphenylhydrazine; and other depositor-supplied synonyms and acronyms (OEHHA, 2021; NCBI, 2021)

1.1.2 Structural and molecular information

Relative molecular mass: 184.24 (IFA, 2021)

Chemical structure:



Molecular formula: $C_{12}H_{12}N_2$

1.1.3 Chemical and physical properties

Description: colourless crystalline solid or powder, colourless in solution; the compound colour may change to yellow or orange owing to the oxidative formation of azobenzene (ATSDR, 2020; IFA, 2021; NCBI, 2021)

Melting point: 123–126 °C (IFA, 2021)

Boiling point: 309 °C, decomposes (NCBI, 2021)

Relative density: 1.16 at 16 °C (IFA, 2021)

Lower explosion limit: 15 g/m³ (IFA, 2021)

Vapour pressure: 4.4×10^{-4} hPa at 25 °C (NCBI, 2021)

Solubility: poorly soluble in water (221 mg/L at 25 °C) (Kühne et al.,1995; IFA, 2021); insoluble in acetic acid, slightly soluble in benzene and dimethyl sulfoxide, very soluble in ethanol (NCBI, 2021)

Octanol/water partition coefficient (P): log K_{ow} = 2.94 (IFA, 2021)

Decomposition temperature: 131 °C (IFA, 2021)

Reactivity: decomposes to aniline and azobenzene at its melting point and above; readily and dangerously reacts with strong oxidizing agents and acids, acid chlorides, and acid anhydrides; autoxidizes in air; rearranges to

benzidine in strong mineral acid (IFA, 2021; NCBI, 2021).

1.1.4 Impurities

Technical-grade 1,2-diphenylhydrazine has considerable levels of impurities and may contain benzidine as a contaminant. In 1 of 16 samples from a manufacturer's continuous production, 25 µg of benzidine per 1 g of 1,2-diphenylhydrazine [equals 25 ppm] was reported (NCBI, 2021).

1.2 Production and use

1.2.1 Production process

1,2-Diphenylhydrazine is produced by the chemical reduction of nitrobenzene in an alkaline medium. Various reducing agents may be used in the process, the most common being iron or zinc metal powders (ATSDR, 2020; NCBI, 2021). After synthesis, separation of 1,2-diphenylhydrazine may be performed with solvent extraction or crystallization in an alcoholic solution (Hallie, 1949; ATSDR, 2020).

1.2.2 Production volume

In 1977, the USA produced at least 450 000 kg of 1,2-diphenylhydrazine and imported 72 100 kg. In 1982, the USA imported 23 200 kg of 1,2-diphenylhydrazine (NTP, 2016). [The Working Group noted that no information was available on current production in the USA or European Union (EU).] The substance is not registered under the REACH Regulation (Registration, Evaluation and Authorization of Chemicals of the European Union), suggesting that less than 1 tonne is manufactured in and/or imported to the European Economic Area (ECHA, 2021). The ChemicalBook database lists more than 100 global suppliers of 1,2-diphenylhydrazine and 3 manufacturers in China (ChemicalBook, 2017) [but it is unknown whether production is continuous or on-demand].

1.2.3 Uses

Historically, 1,2-diphenylhydrazine was widely used globally as a chemical precursor to produce benzidine-based dyes, which were mostly used in the textile industry (NTP, 2016; ATSDR, 2020). Starting from the late 1970s, countries began limiting the manufacture and use of benzidine-based dyes owing to reports of increased incidence of bladder cancer associated with exposure to benzidine (Dapson, 2009). Also in the late 1970s, major dye manufacturers in the USA began phasing out benzidine-based dyes, and production in the USA ceased by 1988 (Dapson, 2009). [The Working Group noted that in developing countries such as China, 1,2-diphenylhydrazine use may be ongoing in some dye- and textile-manufacturing facilities. Textile products imported to Europe occasionally contain benzidine or its derivatives (Piccinini et al., 2008), which are prohibited in the EU (Council Directive 2002/61/EC; European Council, 2002). These chemicals originate from benzidine-based dyes, which can be produced using 1,2-diphenylhydrazine.]

1,2-Diphenylhydrazine has additional uses in the pharmaceutical industry as a chemical intermediate in the manufacture of phenylbutazone (an anti-inflammatory medication) and sulfinpyrazone (a uricosuric medication) (ATSDR, 2020). Although both drugs are now very rarely used in humans in the USA and other developed countries, phenylbutazone is frequently used in veterinary medicine, particularly for treating lameness in horses (Worboys & Toon, 2018).

1.3 Detection and quantification

1.3.1 Air, water, soil, sediment, and other media

No standardized sampling and analytic protocols are available for 1,2-diphenylhydrazine in air, water, soil, sediment, and other media. United States Environmental Protection Agency (US EPA) Method 625.1 is the standard protocol for detecting benzidine and other semi-volatile organic pollutants qualitatively and quantitatively in environmental samples (US EPA, 2016). US EPA Method 625.1 lists 1,2-diphenylhydrazine as a potential "additional extractable analyte", but noted that quantitative determination may be difficult for chemicals in this category. In this method and other published works, 1,2-diphenylhydrazine is extracted from an environmental or product sample with a solvent, and then detection and quantification are carried out using chromatographic and mass spectrometry methods (US EPA, 2016; ATSDR, 2020).

1.3.2 Biological specimens

No specific human biomarker has been identified for detecting and quantifying exposure to 1,2-diphenylhydrazine.

1.4 Occurrence and exposure

1.4.1 Occurrence in the environment, food, and consumer products

The US EPA Toxics Release Inventory (TRI) documented small quantities of 1,2-diphenylhydrazine released into air, surface water, and landfills. Total annual releases from 1998 to 2019 were mostly less than 25 pounds [11.3 kg], except for a landfill release of 260 pounds [118 kg] in 2001 and an air release of 48 pounds [22 kg] in 2017 (US EPA, 2021a).

According to water quality data from the United States National Water Quality Monitoring Council (NWQMC) and summarized by the Agency for Toxic Substances and Disease Registry (ATSDR), 1,2-diphenylhydrazine was detected in 92 of 2409 groundwater samples collected between 1990 and 2020 at concentrations ranging from 0.12 to 21 ppb [µg/L]. Over the same period, 1,2-diphenylhydrazine was detected in 14 of 3286 samples of surface water, with detected concentrations ranging from < 0.2 to 260 ppb [µg/L], and in 3 of 1238 sediment samples, with detected concentrations ranging from < 340 to < 1700 ppb [µg/L] (ATSDR, 2020).

In a national wastewater survey published in 1985, 1,2-diphenylhydrazine was detected in 1.2% of 1205 effluent samples, with a median concentration of < 10 µg/L (Staples et al., 1985). According to a survey of 50 public water-treatment plants by the US EPA and published in 1982, 1,2-diphenylhydrazine was detected in 10 of 347 samples of influent wastewater, with a concentration range of 1–50 µg/L, and in 5 of 362 effluent samples, with a concentration range of 1–2 µg/L (US EPA, 1982).

In other media, 1,2-diphenylhydrazine was screened for but not detected in two studies screening for pollutants in fish caught in the Great Lakes or nearby tributaries, USA (De Vault, 1985; Camanzo et al., 1987). Medications produced using 1,2-diphenylhydrazine (i.e. phenylbutazone and sulfinpyrazone) may contain trace concentrations of the compound (Matsui et al., 1983); however, in the USA and other developed countries, use of these drugs in human medicine has either been discontinued or is very limited (Worboys & Toon, 2018).

[The Working Group noted that 1,2-diphenylhydrazine is difficult to detect and quantify in environmental samples owing to its rapid oxidation during both sample storage and analysis (ATSDR, 2020). Thus, reported concentrations may include azobenzene or 1,2-diphenylhydrazine as degradation products from

other compounds. It was further noted that no data were available on environmental, dietary, or exposure to consumer products outside of the USA.]

1.4.2 Occupational exposure

Workers involved in the manufacture of 1,2-diphenylhydrazine, benzidine-based dyes, phenylbutazone, and sulfinpyrazone may be exposed via ingestion and inhalation of 1,2-diphenylhydrazine. The 1983 United States National Occupational Exposure Survey estimated that 977 workers, including 154 women, were potentially exposed to 1,2-diphenylhydrazine in seven facilities (NIOSH, 2018). [The Working Group noted that the National Occupational Exposure Survey was performed nearly 40 years ago and the estimates reported may not be representative of current exposures.] In the Finnish national register of workers exposed to carcinogenic substances and processes, one chemist was identified as having exposure to 1,2-diphenylhydrazine in 2013 and 2014 (Saalo et al., 2016a, b). No other published studies and reports were available to assess current and historical occupational exposures to 1,2-diphenylhydrazine.

1.4.3 Exposure of the general population

Owing to limited use and release as well as relatively rapid environmental degradation, general population exposure to 1,2-diphenylhydrazine is expected to be low. [The Working Group noted that exposure may have been higher historically for populations living near dye- and textile-production sites when 1,2-diphenylhydrazine was more widely used, or for patients who used phenylbutazone.] However, no specific studies or reports were available to further characterize current and historical exposures in the general population.

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

The United States National Recommended Water Quality Criteria contain two guideline values for 1,2-diphenylhydrazine: 0.03 µg/L for human health, for the consumption of water and aquatic organisms; and 0.2 µg/L for human health, for the consumption of aquatic organisms only (US EPA, 2021b). The Australian and New Zealand Guidelines for Fresh and Marine Water Quality recommended 2 µg/L in fresh and marine water as a toxicant default guideline value for protecting aquatic ecosystems (Water Quality Australia, 2020).

The United States ATSDR derived an intermediate duration minimal risk level (MRL) of 0.05 mg/kg per day for oral intake of 1,2-diphenylhydrazine on the basis of hepatic toxicity in rats (ATSDR, 2020). No other MRLs were derived for acute or chronic oral intake, or for other exposure routes owing to limited data.

According to the harmonized classification and labelling implemented in the European Union (Classification, Labelling and Packaging Regulation, 1272/2008/EC), 1,2-diphenylhydrazine has the following classification: carcinogen, category 1B; acute toxicity, category 4; acute aquatic toxicity, category 1; and chronic aquatic toxicity, category 1. Because of this classification, this substance is subject to several other European Union regulations (e.g. general product safety, food contact, medical devices) (ECHA, 2021). Employers are obliged under the Classification, Labelling and Packaging Regulation to minimize worker exposure to 1,2-diphenylhydrazine and must arrange for medical surveillance of exposed workers (Council Directive 98/24/EC; European Council, 1998). European Union Council Directives state that pregnant or breastfeeding workers and persons under age 18 years may not be occupationally exposed to 1,2-diphenylhydrazine (Council directive 92/85/EEC;

Council directive 94/33/EC; European Council, 1992, 1994).

1.5.2 Reference values for biological monitoring of exposure

No reference value was available for biological monitoring of exposure to 1,2-diphenylhydrazine in humans.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

3.1.1 Oral administration (feed)

In a well-conducted study by the United States National Cancer Institute (NCI), groups of 50 male and 47–50 female B6C3F$_1$ mice (age, 6 weeks) were given feed containing technical-grade hydrazobenzene [1,2-diphenylhydrazine] [purity not reported; one unidentified impurity] (NTP, 1978). The experiments at the higher and lower doses were conducted separately, each dose group having its own controls for males and females (50 males and 50 females per group). The time-weighted average (TWA) dietary concentrations used were 0%, 0.008%, and 0.04% for males and 0%, 0.004%, and 0.04% for females in the control groups and at the lower and higher doses, respectively. After 78 weeks of treatment with 1,2-diphenylhydrazine, observation of the mice continued for an additional 17 weeks (lower dose) or 18 weeks (higher dose). The study was terminated at week 95–96. Survival of male and female mice was significantly lower for the group at the higher dose than for the controls. However, there were adequate numbers of animals at risk for late-developing tumours, with survival at study termination being 66% (33/50) for males at the higher dose compared with 78% (39/50) for the respective control group, and 52% (26/50) for females at the higher dose compared with 76% (38/50) for the respective control group. In mice at the lower dose, survival at study termination was 88% (44/50) for males compared with 86% (43/50) for males in the respective control group, and 79% (37/47) for females compared with 72% (36/50) for females in the respective control group. No distinct pattern of mean body-weight change was evident in groups of males and females at the lower dose. After week 28, a decrease in mean body weight compared with controls was observed for male and female mice at the higher dose [read from the figure, the decreases reached approximately 30% at week 78 of administration]. All mice underwent complete necropsy, and histopathology was performed on major tissues, organs, and gross lesions taken from killed animals and, whenever possible, from animals found dead.

In female mice, there was a significant increase in the incidence of hepatocellular carcinoma ($P < 0.001$, Fisher exact test) at the higher dose but not at the lower dose (higher dose, 20/43 versus 1/50 in controls; lower dose, 4/39 versus 2/47 in controls). The incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased ($P < 0.001$, Fisher exact test) at the higher dose (22/43 versus 1/50 in controls), but not at the lower dose (4/39 versus 2/47 in controls). 1,2-Diphenylhydrazine did not significantly increase the incidence of any tumours in treated male mice. [The Working Group noted that this was a well-described and well-conducted study, using two doses in both males and females (with respective control groups), with an adequate number of animals per group. The lack of data confirming the purity and stability of 1,2-diphenylhydrazine in the dosed feed was considered a

Table 3.1 Studies of carcinogenicity in experimental animals exposed to 1,2-diphenylhydrazine

Study design Species, strain (sex) Age at start Duration Reference	Route, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) ~6 wk 95 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.008% diet, ad libitum for 78 wk 50, 50 43, 44	No significant increase in tumour incidence in treated animals		Principal strengths: males and females used; well-conducted study; adequate number of mice per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA
Full carcinogenicity Mouse, B6C3F$_1$ (M) ~6 wk 95–96 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.04% diet, ad libitum for 78 wk 50, 50 39, 33	No significant increase in tumour incidence in treated animals		Principal strengths: males and females used; well-conducted study; adequate number of mice per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA
Full carcinogenicity Mouse, B6C3F$_1$ (F) ~6 wk 95–96 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.004% diet, ad libitum for 78 wk 50, 47 36, 37	No significant increase in tumour incidence in treated animals		Principal strengths: males and females used; well-conducted study; adequate number of mice per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA
Full carcinogenicity Mouse, B6C3F$_1$ (F) ~6 wk 96 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.04% diet, ad libitum for 78 wk 50, 50 38, 26	*Liver* Hepatocellular carcinoma Tumour incidence: 1/50, 20/43* Hepatocellular adenoma or carcinoma (combined) Tumour incidence: 1/50, 22/43*	*$P < 0.001$, one-tailed Fisher exact test *$P < 0.001$, one-tailed Fisher exact test	Principal strengths: males and females used; well-conducted study; adequate number of mice per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Mouse, strain A (M) 6–8 wk 24 wk Maronpot et al. (1986)	Intraperitoneal injection Purity, NR Tricaprylin 0, 50, 100, 200 mg/kg bw 3×/wk for 8 wk 60, 10, 10, 10 54, 10, 8, 9	*Lung*: pulmonary tumours Tumour incidence: 7/54, 1/10, 0/8, 6/9* Tumour multiplicity: 0.167, 0.10, 0.00, 0.89*	*$P < 0.05$, Fisher exact test *$P < 0.05$, *t*-test	Principal strengths: used males and females. Other comments: histological characterization of "pulmonary tumours" was not reported
Full carcinogenicity Mouse, strain A (F) 6–8 wk 24 wk Maronpot et al. (1986)	Intraperitoneal injection Purity, NR Tricaprylin 0, 50, 100, 200 mg/kg bw 3×/wk for 8 wk 60, 10, 10, 10 54, 10, 10, 9	*Lung*: pulmonary tumours Tumour incidence: 6/54, 2/10, 2/10, 3/9 Tumour multiplicity: 0.110, 0.30, 0.30, 0.56*	NS *$P < 0.05$, *t*-test	Principal strengths: used males and females. Other comments: histological characterization of "pulmonary tumours" was not reported
Full carcinogenicity Rat, F344 (M) ~6 wk 107–108 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.008% diet, ad libitum for 78 wk 50, 50 35, 39	*Liver* Hepatocellular carcinoma Tumour incidence: 0/47, 5/49* Neoplastic nodules or hepatocellular carcinoma (combined) Tumour incidence: 5/47, 13/49*	*$P = 0.031$, one-tailed Fisher exact test *$P = 0.040$, one-tailed Fisher exact test	Principal strengths: used males and females; well-conducted study; adequate number of rats per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Rat, F344 (M) ~6 wk 106–109 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.03% diet, ad libitum for 78 wk 49, 50 36, 32	*Liver* Hepatocellular carcinoma Tumour incidence: 1/48, 31/49*	*$P < 0.001$, one-tailed Fisher exact test	Principal strengths: used males and females; well-conducted study; adequate number of rats per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA
		Neoplastic nodules or hepatocellular carcinoma (combined) Tumour incidence: 1/48, 37/49*	*$P < 0.001$, one-tailed Fisher exact test	
		Adrenal gland: pheochromocytoma or malignant pheochromocytoma (combined) Tumour incidence: 8/47, 16/46*	*$P = 0.042$, one-tailed Fisher exact test	
		Zymbal gland: squamous cell carcinoma Tumour incidence: 0/48, 5/49*	*$P = 0.030$, one-tailed Fisher exact test	
		Ear canal, Zymbal gland, or skin of the ear: squamous cell carcinoma or squamous cell papilloma (combined) Tumour incidence: 0/48, 7/49*	*$P = 0.007$, one-tailed Fisher exact test	
Full carcinogenicity Rat, F344 (F) ~6 wk 108–109 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.004% diet, ad libitum for 78 wk 50, 50 39, 37	No significant increase in tumour incidence in treated animals		Principal strengths: used males and females; well-conducted study; adequate number of rats per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Rat, F344 (F) ~6 wk 107–109 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.01% diet, ad libitum for 78 wk 50, 50 43, 25	*Liver*: neoplastic nodules Tumour incidence: 0/50, 6/50* *Mammary gland*: adenocarcinoma, NOS Tumour incidence: 0/50, 6/50*	*P = 0.013, one-tailed Fisher exact test *P = 0.013, one-tailed Fisher exact test	Principal strengths: used males and females; well-conducted study; adequate number of rats per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA; historical controls – mammary gland adenocarcinoma: 8/585 (1.4%)

bw, body weight; F, female; M, male; NOS, not otherwise specified; NR, not reported; NS, not significant; TWA, time-weighted average; wk, week.

limitation of this study. The Working Group also noted possible contamination of technical-grade 1,2-diphenylhydrazine with benzidine at concentrations up to 25 ppm (see Section 1.1.4). Although oral administration of benzidine (in the feed) has been shown to cause hepatocellular neoplasms in male and female B6C3F$_1$ mice (IARC, 2012), the dose level at which benzidine was carcinogenic was considerably higher than that reported in the present study (NTP, 1978) had the carcinogenic response been attributable to benzidine as a contaminant of 1,2-diphenylhydrazine. A difference between 1,2-diphenylhydrazine and benzidine in terms of carcinogenic response elicited was that 1,2-diphenylhydrazine caused hepatocellular neoplasms only in female B6C3F$_1$ mice.]

3.1.2 Skin application

In a lifetime carcinogenicity study in CC57 brown mice (Pliss, 1974), 2 mg of 1,2-diphenylhydrazine [purity not reported] dissolved in 0.05 mL of benzene was applied to the skin (of the interscapular region) of 50 male and female mice (age, 2 months) [sex distribution not reported], three times per week for 63 weeks. The total dose of 1,2-diphenylhydrazine per mouse was 360 mg. At 25 weeks, when the first tumour (a squamous cell carcinoma of the skin) was observed, survival was 41/50 (12 females and 29 males). Survival was 16/50 (3 females and 13 males) after 1 year, and only 4/50 (all males) after 1.5 years. All mice underwent complete necropsy, and main organs and tissues [Working Group assumption] were evaluated by histopathology.

No tumours developed at the site of application, but nine mice developed systemic tumours: one mouse developed a squamous cell carcinoma of the skin, two developed liver haemangioma, three developed leukaemia, and three developed lung adenoma. Two mice developed two tumours simultaneously: the first mouse developed liver adenoma and lung adenoma, and the second mouse developed liver adenoma and leukaemia. [The Working Group noted the lack of controls, the use of benzene as the vehicle, the lack of information on body weight, the small number of animals per group, and the fact that the number of animals per sex was not reported; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

3.1.3 Intraperitoneal injection

In a carcinogenicity study involving blind testing of 65 chemicals (Maronpot et al., 1986), groups of 10 male and 10 female strain A mice (age, 6–8 weeks) were treated with 1,2-diphenylhydrazine ([purity not reported]; dissolved in tricaprylin) at a dose of 50, 100, or 200 mg per kg body weight (bw) for the groups at the lowest, intermediate, and highest dose, respectively, by intraperitoneal injection (0.1 mL), three times per week for 8 weeks. [The Working Group considered that the short study duration and low number of animals per group were adequate for this strain of mice, which has a high incidence of spontaneous pulmonary neoplasms.] Groups of 60 male and 60 female tricaprylin-injected (0.1 mL) mice served as vehicle controls. After 8 weeks, the mice were held for 16 weeks until the end of the experiment. The highest dose level of 1,2-diphenylhydrazine used was chosen because this dose did not cause death, growth retardation, or overt toxicity in a preliminary dose-setting study.

A significant increase in the incidence of pulmonary tumours ($P < 0.05$, Fisher exact test) was observed in males at the highest dose compared with vehicle controls, and a significant increase in the multiplicity (ratio of tumours per mouse) of pulmonary tumours ($P < 0.05$, *t*-test) was observed in males and females at the highest dose compared with their respective controls. In these groups at the highest dose, the proportion of male survivors with tumours was 67% [6/9],

and tumour multiplicity was 0.89, while the proportion of female survivors with tumours was 33% [3/9] and tumour multiplicity was 0.56. In each of the groups of females at the lowest and intermediate dose, which each included 10 survivors, two mice developed pulmonary tumours; while in the group of males at the lowest dose, which included 10 survivors, one mouse developed pulmonary tumours; however, the differences were not significant compared with vehicle controls. The incidence and multiplicity of pulmonary tumours in mice in the vehicle-control groups were 7/54 and 0.167, respectively, in males, and 6/54 and 0.110, respectively, in females. [The Working Group noted the use of males and females, and that the histological characterization of "pulmonary tumours" was not reported, hence, the designation of the tumours as benign (adenoma) or malignant (carcinoma) was not possible.]

3.1.4 Subcutaneous injection

In a lifetime carcinogenicity study, 60 CC57 brown mice (age, 2 months; [sex distribution not reported]) were subcutaneously injected with a suspension containing 5 mg of 1,2-diphenylhydrazine [purity not reported] in 0.2 mL of sunflower oil, once per week for 73 weeks (Pliss, 1974). The first tumour (a rhabdomyosarcoma at the site of injection) was observed at week 38, when 29 males and 1 female were still alive. At 1 year after the first injection, 22 mice survived. Only 3 mice were alive at 1.5 years. All mice underwent complete necropsy, and main organs and tissues [Working Group assumption] were evaluated by histopathology.

Overall, 11 mice developed tumours (2 mice developed rhabdomyosarcoma at the site of injection, 5 mice developed lung adenoma, 1 mouse developed liver adenoma, 2 mice developed liver haemangioma, and 1 mouse developed leukaemia). One mouse developed both a rhabdomyosarcoma and a liver haemangioendothelioma; another mouse developed both a lung adenoma and a liver haemangioma. Thus, 36.7% (11/30) of mice treated with 1,2-diphenylhydrazine by subcutaneous infection developed tumours. In the control group [not further specified], only 17% of the animals developed spontaneous tumours, which the authors stated were of a type and morphology analogous to the tumours induced by 1,2-diphenylhydrazine. [The Working Group noted that tumours in the control group were unspecified, and no information on body weight was provided; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

3.2 Rat

3.2.1 Oral administration (feed)

In a lifetime carcinogenicity study, 20 male Wistar rats (age, 6–8 weeks) were given feed ("Larsen's diet", a synthetic feed) containing 1,2-diphenylhydrazine [purity not reported] at a TWA dose of 2 mg per day until spontaneous death occurred (Marhold et al., 1968). An untreated control group of 50 male Wistar rats received feed only. Average duration of survival and average body weight at the end of the experiment were lower in the group treated with 1,2-diphenylhydrazine (288 days and 121 g, respectively) than in the control group (378 days and 141 g, respectively). No tumours were observed in either the group treated with 1,2-diphenylhydrazine or in the control group. [The Working Group noted the small number of treated animals and the limited reporting of experimental details, including information on postmortem examination and histopathological evaluation; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

In a lifetime carcinogenicity study, 72 male and female outbred rats [age at start and sex distribution not reported], were given feed supplemented with 30 mg of 1,2-diphenylhydrazine [purity not reported] in 0.5 mL of sunflower oil, 5 times per week for 84 weeks (Pliss, 1974). The mean total dose of 1,2-diphenylhydrazine was 12.57 g per rat. After 1 year, 42 rats survived (25 females and 17 males). All mice underwent complete necropsy, and main organs and tissues [Working Group assumption] were evaluated by histopathology.

Overall, 21 rats (16 females and 5 males) developed tumours: 5 rats developed tumours of the Zymbal gland (malignant in two cases, and bilateral in one case); 6 rats developed liver tumours (including 4 hepatic adenomas, 1 cholangioma, and 1 lymphangioma); 4 rats developed mammary gland tumours (2 adenocarcinomas and 2 fibroadenomas); 3 rats developed uterine tumours (1 adenocarcinoma, 1 lymphangioma, and 1 lymphangiosarcoma); 2 rats developed kidney tumours (1 multiple papillary adenoma, and 1 bilateral tubulo-papillary adenocarcinoma associated with hepatocellular carcinoma and skin fibroma); and 1 rat developed a lymphoid leukaemia (Pliss, 1974). [The Working Group noted the lack of a control group, and the fact that sex distribution at start and bodyweight data were not reported; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

In a well-conducted study by the NCI, groups of 49–50 male and 50 female Fischer 344 rats (age, 6 weeks) were given feed containing technical-grade hydrazobenzene [1,2-diphenylhydrazine] [purity not reported; one unidentified impurity] (NTP, 1978). The experiments at the higher dose and at the lower dose were conducted separately, each dose group having its own controls for males and females (49–50 males and 49–50 females per group). The TWA dietary concentrations used were 0%, 0.008%, and 0.03% for males, and 0%, 0.004% and 0.01% for females in the control groups and at the lower and higher doses, respectively. After 78 weeks of treatment with 1,2-diphenylhydrazine, observation of the rats continued for an additional 28–30 weeks. The study was terminated at week 106–109. Survival of female rats was significantly lower in the group at the higher dose than in the controls, but survival of male rats was not influenced by treatment with 1,2-diphenylhydrazine. For rats at the higher dose, survival at study termination was 64% (32/50) for males compared with 73% (36/49) for males in the respective control group, and 50% (25/50) for females compared with 86% (43/50) for females in the respective control group. For rats at the lower dose, survival at study termination was 78% (39/50) for males compared with 70% (35/50) for males in the respective control group, and 74% (37/50) for females compared with 78% (39/50) for females in the respective control group. A slight decrease in mean body weight relative to controls was apparent for males at the higher dose, but not for males at the lower dose. A slight decrease in mean body weight was also observed for females at the lower dose after week 46, and for females at the higher dose after week 22. All rats underwent complete necropsy, and full histopathology was performed on major tissues, organs, and gross lesions taken from killed animals and, whenever possible, from animals found dead.

The incidence of hepatocellular carcinoma was significantly increased in males at the lower dose (5/49; $P = 0.031$, Fisher exact test) and at the higher dose (31/49; $P < 0.001$, Fisher exact test), compared with their control groups (0/47 and 1/48, respectively). There was also a significant increase in the incidence of liver neoplastic nodules or hepatocellular carcinoma (combined) in males at the lower dose (13/49; $P = 0.040$, Fisher exact test) and at the higher dose (37/49; $P < 0.001$, Fisher exact test), compared with their control groups (5/47 and 1/48, respectively). A significant increase in the incidence of pheochromocytoma

or malignant pheochromocytoma (combined) of the adrenal gland (16/46; $P = 0.042$, Fisher exact test) was also observed in males at the higher dose compared with males in the respective control group (8/47). There were significant increases in the incidence of squamous cell carcinoma of the Zymbal gland (5/49; $P = 0.030$, Fisher exact test), and of squamous cell papilloma or carcinoma (combined) of the ear canal, Zymbal gland or skin of the ear (combined) (7/4; $P = 0.007$, Fisher exact test) in males at the higher dose compared with males in the respective control group (0/48 and 0/48, respectively).

In females, there was a significant increase in the incidence of mammary gland adenocarcinoma (not otherwise specified) (6/50 for the group at the higher dose versus 0/50 for the respective control group; $P = 0.013$, Fisher exact test), and in the incidence of neoplastic nodules in the liver (6/50 for the group at the higher dose versus 0/50 for the respective control group; $P = 0.013$, Fisher exact test). [The Working Group noted that this was a well-described and well-conducted study, using two doses in both males and females (with respective control groups), with an adequate number of animals per group. The lack of data confirming the stability of 1,2-diphenylhydrazine in dosed feed was a limitation of this study. The Working Group also noted possible contamination of technical-grade 1,2-diphenylhydrazine with benzidine at concentrations up to 25 ppm (see Section 1.1.4). Although oral administration (by gavage) of benzidine has also been shown to cause mammary gland carcinomas in female Sprague-Dawley rats (IARC, 2012), no hepatocellular neoplasms were observed, unlike the carcinogenic response in this study with 1,2-diphenylhydrazine (NTP, 1978).]

3.2.2 Subcutaneous injection

In a lifetime carcinogenicity study, a group of 52 Sherman female rats (age, ~10 weeks) was treated with 60 mg of technical-grade 1,2-diphenylhydrazine [purity not reported] in olive oil (1 mL) by subcutaneous injection, once per week, for life (Spitz et al., 1950). A control group of 50 rats was treated with olive oil (1 mL) only. The rats were killed if they showed dramatic loss of weight or obvious illness. The survival rate of rats treated with 1,2-diphenylhydrazine was the same as that of the control group. Rats in both the treated group and the control group (22%) died during the first 200 days of the experiment during a period of hot weather when the rooms were not air-conditioned. Rats in the control group additionally died from tracheobronchitis with associated abscesses of the lung, otitis, meningitis, and brain abscess. The body weights of control and treated rats were similar and reached a maximum of 250–280 g. All rats underwent complete necropsy.

No hepatic tumours developed in treated rats. One keratinized squamous cell carcinoma (1/52) occurred on the skin of the external auditory canal of a treated rat. [The Working Group noted the inadequacy of the conditions of animal maintenance, the lack of reporting results for controls, and the lack of details on postmortem examination and histopathological evaluation; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

In a lifetime carcinogenicity study, 91 male and female outbred rats [age at start and sex distribution not reported] were given 60 mg of 1,2-diphenylhydrazine [purity not reported] in 0.3–0.5 mL of sunflower oil by subcutaneous injection, once per week, for 10 weeks. The dose was then lowered to 40 mg per week until the end of the exposure (84 weeks) (Pliss, 1974). All rats underwent complete necropsy, and main organs and tissues [Working Group assumption] were evaluated by histopathology.

The first tumour (Zymbal gland carcinoma) appeared at week 27, when 29 females and 24 males were still alive. After 1 year, 22 females and 15 males were still alive. The last rat with

a tumour (liver adenoma) died 98 weeks after the beginning of the experiment. Mean tumour latency was 74 weeks, and tumours developed in 12 rats: three rats developed mammary gland tumours (two with microfollicular cancers, and one with adenoma and fibroadenoma); three rats developed liver tumours (including one malignant adenoma [carcinoma] and one adenoma associated with a pulmonary lymphosarcoma); three rats developed Zymbal gland tumours (one basalioma [basal cell carcinoma], one carcinoma, one squamous cell carcinoma); one rat developed a uterine adenocarcinoma, one rat developed a haemangioma of the spleen, and one rat developed reticulosis. [The Working Group noted the lack of controls and that the number of animals per sex was not reported; therefore, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

In a lifetime carcinogenicity study, a group of 50 outbred rats (25 males and 25 females) ([age at start not reported]; body weight, 100–120 g), were exposed by subcutaneous injection to 20 mg of a paste that contained 55% 1,2-diphenylhydrazine [purity not reported], 15% zinc compounds, and 30% water (dissolved in 0.5 mL of sunflower oil), once per week for 1 year (Genin at al., 1975; Shabad & Genin, 1975; also reported in Kurliandskiĭ et al., 1976). A group of 50 control animals (25 males and 25 females) received sunflower oil only. All rats underwent complete necropsy and main organs and tissues [Working Group assumption] were evaluated by histopathology.

The survival rate until the time of onset of the first tumour (~86 weeks) in the group treated with 1,2-diphenylhydrazine was almost half that of controls (19/50 compared with 36/50). No hepatic tumours were observed in treated rats, but there was one polymorphic cell sarcoma at the site of injection, one squamous cell carcinoma of the Zymbal gland, one endometrial polyposis, and one anaplastic (embryonal) kidney cancer. Mean tumour latency in the group treated with 1,2-diphenylhydrazine was 86 ± 6 weeks. In the control group, one fibroadenoma of the mammary gland was detected, with a latent period of 90 weeks. [The Working Group noted that the body weights of control and treated animals, the composition of the feed, and rates of food consumption were not reported, and the time of occurrence of tumours was unspecified; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of 1,2-diphenylhydrazine has been assessed in one well-conducted study in male and female B6C3F$_1$ mice and one well-conducted study in male and female Fischer 344 rats treated by oral administration (in the feed) (NTP, 1978), in two additional studies in male Wistar rats (Marhold et al., 1968) and in male and female outbred rats (Pliss, 1974) also treated by oral administration (in the feed); in male and female strain A mice treated by intraperitoneal injection (Maronpot et al., 1986); in male and female CC57 brown mice treated by skin application (Pliss, 1974); and in three studies in male and female CC57 brown mice (Pliss, 1974), in female Sherman rats (Spitz et al., 1950), and in male and female outbred rats (Pliss, 1974; Genin et al., 1975) treated by subcutaneous injection.

In the well-conducted study in male and female B6C3F$_1$ mice treated by oral administration, there was a significant increase in the incidence of hepatocellular carcinoma and of hepatocellular adenoma or carcinoma (combined) in female mice at the higher dose, but not at the lower dose, compared with their respective controls. There were no significant effects upon the incidence of neoplasms in treated male mice (NTP, 1978).

In the well-conducted study in male and female Fischer 344 rats treated by oral administration, the incidence of hepatocellular carcinoma was significantly increased in males at the lower and higher dose, compared with their respective controls. In male rats, there was also a significant increase in the incidence of liver neoplastic nodules or hepatocellular carcinoma (combined) at the lower and higher dose, compared with their respective controls. A significant increase in the incidence of pheochromocytoma or malignant pheochromocytoma (combined) of the adrenal gland was also observed in males at the higher dose compared with controls. There were significant increases in the incidence of squamous cell carcinoma of the Zymbal gland, and of squamous cell papilloma or carcinoma (combined) of the ear canal, Zymbal gland or skin of the ear (combined) in males at the higher dose compared with controls. In female rats, there was a significant increase in the incidence of mammary gland adenocarcinoma (not otherwise specified), and in the incidence of neoplastic nodules in the liver, both at the higher dose (NTP, 1978).

In the study in male and female strain A mice treated by intraperitoneal injection, a significant increase in the incidence of pulmonary tumours was observed in male mice at the higher dose, and a significant increase in the multiplicity (tumour per mouse ratio) of pulmonary tumours was observed in male and female mice at the higher dose compared with their respective controls (Maronpot et al., 1986).

Two studies in male and female Wistar rats (Marhold et al., 1968) and in male and female outbred rats (Pliss, 1974) treated by oral administration (in the feed), one study in male and female CC57 brown mice treated by skin application (Pliss, 1974), one study in male and female CC57 brown mice treated by intraperitoneal injection (Pliss, 1974), and three studies in male and female CC57 brown mice (Pliss, 1974), in female Sherman rats (Spitz et al., 1950), and in male and female outbred rats (Pliss, 1974; Genin et al., 1975) treated by subcutaneous injection were judged to be inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

No data were available to the Working Group

4.1.2 Experimental systems

(a) Absorption, distribution, and excretion

The only data available on the absorption, distribution, and excretion of 1,2-diphenylhydrazine in laboratory animals were those of Dodd et al. (2012) and Dutkiewicz & Szymanska (1973).

Dodd et al. (2012) detected 1,2-diphenylhydrazine in the blood of male Fischer 344 rats given feed containing 1,2-diphenylhydrazine at 200 or 300 ppm for 13 weeks; mean blood concentrations of 1,2-diphenylhydrazine ranged from 0.002 to 0.006 µg/mL. In rats exposed at ≤ 80 ppm, 1,2-diphenylhydrazine blood concentrations were below the limit of quantitation (approximately 0.001 µg/mL) throughout the study.

In a study by Dutkiewicz & Szymanska (1973) in Wistar rats, 1,2-diphenylhydrazine was administered as a single oral (200 or 400 mg/kg bw), intraperitoneal (100 or 200 mg/kg bw), intravenous (4 or 8 mg/kg bw), or intratracheal (5 or 10 mg/kg bw) dose and urinary metabolites were analysed. Dutkiewicz & Szymanska (1973) noted unchanged 1,2-diphenylhydrazine in the urine in rats treated by any route. In addition, there was an unidentified metabolite in the urine of rats treated by intratracheal and oral administration,

suggesting that some urinary excretion had occurred. [The Working Group noted that it was not clear whether this metabolite was associated with exposure to 1,2-diphenylhydrazine, although this was plausible.]

No data were available regarding the distribution of 1,2-diphenylhydrazine in laboratory animals.

(b) Metabolism

In the study by Dutkiewicz & Szymanska (1973), benzidine and aniline were reported to be metabolites in the urine of Wistar rats exposed to 1,2-diphenylhydrazine. Other metabolites included two unspecified hydroxy derivatives of benzidine, 2-aminophenol and 4-aminophenol, and unidentified compounds. [The Working Group noted that the analytical method used in this study was thin-layer chromatography, which may have produced degradation products that were identified as unchanged 1,2-diphenylhydrazine or metabolites.]

With the exception of the unspecified hydroxy derivatives noted above, chemical metabolites were also noted in several other studies in experimental systems (Williams, 1959; National Research Council, 1981; IARC, 1972). Bolton & Griffiths reported the metabolism of 1,2-diphenylhydrazine to aniline by isolated bacterial microflora from rats (Bolton & Griffiths, 1978). These findings are consistent with the metabolic scheme shown in Fig. 4.1, which is based on data for azobenzene and aniline. [The Working Group noted that the enzymes implicated in the metabolism of 1,2-diphenylhydrazine in rodents have not been identified. The Working Group also noted that, based on the available evidence, the metabolism of 1,2-diphenylhydrazine to aniline and benzidine is plausible but uncertain.]

4.2 Evidence relevant to key characteristics of carcinogens

4.2.1 Is genotoxic

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1.

1,2-Diphenylhydrazine (100 mg/kg bw; [purity not reported]) administered intraperitoneally to male mice induced DNA damage as measured by inhibition of testicular DNA synthesis (thymidine incorporation) (Seiler, 1977), but did not induce hepatic DNA strand breaks, as measured by alkaline elution, in female Sprague-Dawley rats treated with 1,2-diphenylhydrazine at 60 and 130 mg/kg bw by gavage (Kitchin et al., 1994).

(ii) Non-human mammalian cells in vitro

See Table 4.2.

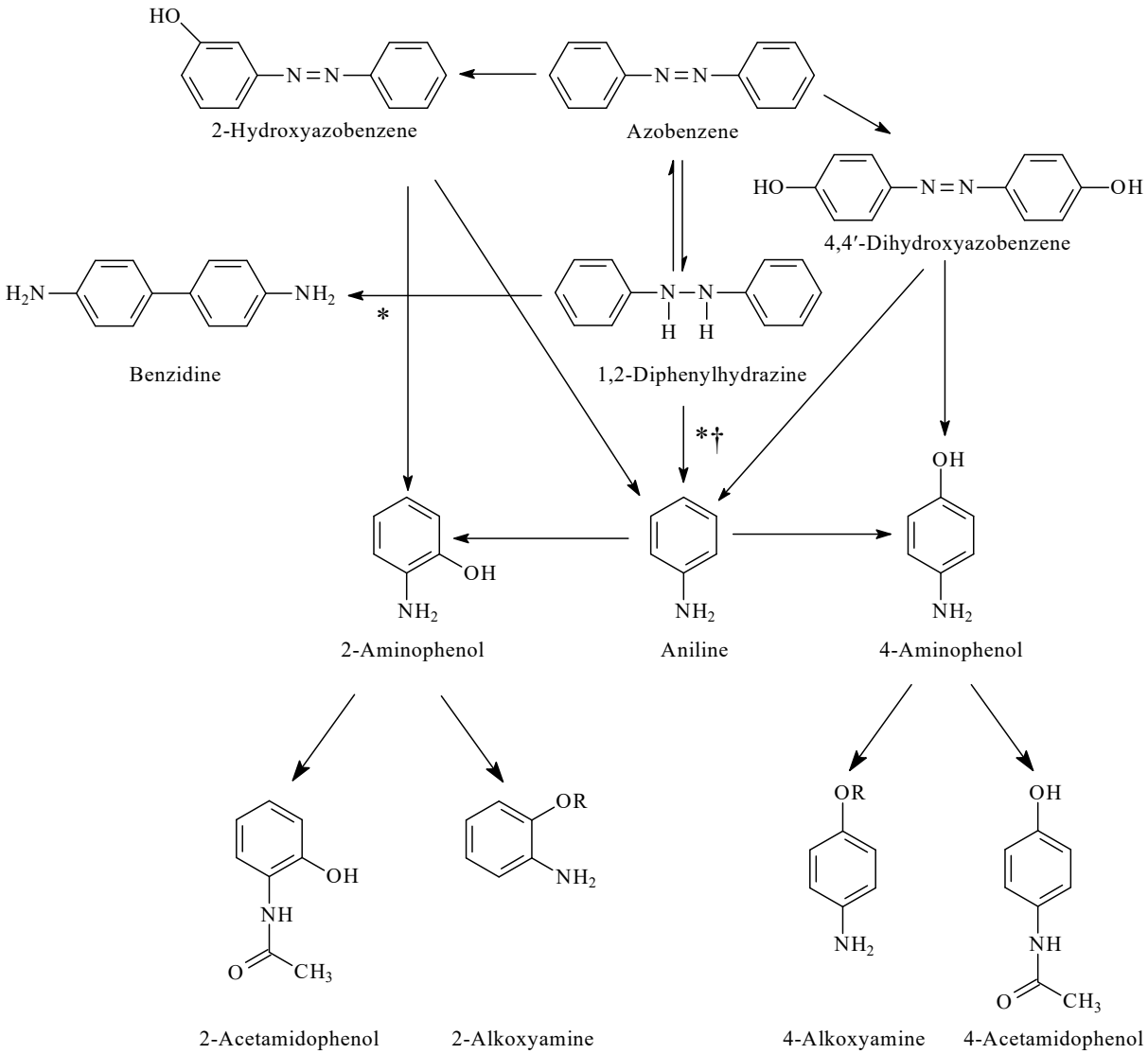
1,2-Diphenylhydrazine induced chromosomal aberrations and sister-chromatid exchanges in the presence of metabolic activation in Chinese hamster ovary (CHO) cells. The response was equivocal for chromosomal aberrations in the absence of metabolic activation (Galloway et al., 1987).

(iii) Non-mammalian experimental systems

See Table 4.3.

1,2-Diphenylhydrazine did not cause sex-linked recessive lethal mutations in *Drosophila melanogaster* (Yoon et al., 1985). 1,2-Diphenylhydrazine induced mutations in *Salmonella typhimurium* strain TA100 with metabolic activation but did not induce mutations in strains TA98, TA1535, TA1537, and TA1538 (Dunkel et al., 1985; Haworth et al., 1983), and gave negative results in the *Escherichia coli* WP2 *uvrA* (Dunkel et al., 1985). [The Working Group

Fig. 4.1 Proposed metabolic scheme for 1,2-diphenylhydrazine



R = SO_3H or $C_6H_9O_6$

* Reported in studies in rats treated with 1,2-diphenylhydrazine (Dutkiewicz & Szymanska, 1973) [The Working Group noted that the analytical method used in this study was thin-layer chromatography, which may have produced degradation products that were identified as unchanged 1,2-diphenylhydrazine or metabolites.]

† Reported in studies using the isolated microflora of rats (Bolton & Griffiths, 1978).

Created by the Working Group.

Table 4.1 Genetic and related effects of 1,2-diphenylhydrazine in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks, alkaline elution	Rat, Sprague-Dawley (F)	Liver	–	60 mg/kg bw, 130 mg/kg bw	Oral (gavage), first dose of 60 mg/kg bw given to one group of rats 21 h before killing; second dose of 130 mg/kg bw given to another group of rats 4 h before killing		Kitchin et al. (1994)
Inhibition of testicular DNA synthesis	Mouse, Swiss (M)	Testes	+	100 mg/kg bw	Single intraperitoneal dose, mice killed 3.5 h later	Purity, NR The assay used has low sensitivity	Seiler (1977)

bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male; NR, not reported.

[a] +, positive; –, negative.

Table 4.2 Genetic and related effects of 1,2-diphenylhydrazine in non-human mammalian cells in vitro

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Chromosome aberrations	Chinese hamster, ovary (CHO) cells	+/–	+	42 µg/mL –S9, 14 µg/mL +S9		Galloway et al. (1987)
Sister-chromatid exchanges	Chinese hamster, ovary (CHO) cells	–	+	14 µg/mL –S9, 14 µg/mL +S9 in one trial (weak positive), 5 µg/mL +S9 in another trial (positive)		Galloway et al. (1987)

HIC, highest ineffective concentration; LEC, lowest effective concentration; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative; +/–, equivocal.

Table 4.3 Genetic and related effects of 1,2-diphenylhydrazine in non-mammalian experimental and acellular systems

Test system species, strain	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Drosophila melanogaster	Sex-linked recessive lethal mutations	NT	– –	50 ppm (oral, 3 days) 80 ppm (injection)		Yoon et al. (1985)
Salmonella typhimurium, TA100	Reverse mutation base-pair substitution	–	+	100 µg/plate –S9, 33.3 µg/plate + RLI S9 and 100 µg/plate + HLI S9	Purity, NR	Haworth et al. (1983)
Salmonella typhimurium, TA98, TA1535, and TA1537	Reverse mutation	–	–	100 µg/plate	Purity, NR	Haworth et al. (1983)
Salmonella typhimurium, TA100	Reverse mutation	–	+	333 µg/plate –S9, 100 µg/plate +S9	Results from four independent laboratories; metabolic activation compared across several systems: rat S9; RLI S9; mouse S9; MLI S9; hamster S9; HLI S9	Dunkel et al. (1985)
Salmonella typhimurium, TA98, TA1535, TA1537, and TA1538	Reverse mutation	–	–	333 µg/plate		
Escherichia coli, WP2 *uvrA*	Reverse mutation	–	–	3333 µg/plate		
DNA fragment from the human c-Ha-*RAS*-1 protooncogene and *TP53* tumour suppressor gene (acellular system)	DNA damage in the presence of copper(II)	+	NT	20 µM [3.7 µg/mL]	Four concentrations tested (20, 30, 40, and 50 µM) with increasing dose–response relationship	Ohnishi et al. (2000)

HIC, highest ineffective concentration; HLI, hamster liver (Aroclor 1254)-induced; LEC, lowest effective concentration; MILI, mouse liver (Aroclor 1254)-induced; NR, not reported; NT, not tested; ppm, parts per million; RLI, rat liver (Aroclor 1254)-induced; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative.

noted that the purity of 1,2-diphenylhydrazine was not reported for the study by Haworth et al. (1983).] Ohnishi et al. (2000) reported that 1,2-diphenylhydrazine induced DNA damage at thymidine residues, in a study using [^{32}P]-5′-end-labelled DNA fragments obtained from the human c-Ha-*RAS*-1 protooncogene and the *TP53* tumour suppressor gene. They reported that the DNA damage was caused by 1,2-diphenylhydrazine in the presence of copper(II), and this was significantly enhanced by treatment with piperidine, suggesting that 1,2-diphenylhydrazine caused base modification and liberation. [The Working Group noted that this genotoxic effect required the presence of copper in an acellular system.]

4.2.2 Evidence relevant to other key characteristics

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Regarding oxidative stress, Ohnishi et al. (2000) reported that 1,2-diphenylhydrazine in the presence of copper(II) caused an increase in the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine, a biomarker of oxidative DNA damage, in calf thymus DNA (and formation of H_2O_2), which suggests that 1,2-diphenylhydrazine induces oxidative stress.

Regarding chronic inflammation, in a study by the NCI (NTP, 1978), groups of male and female Fischer 344 rats and B6C3F$_1$ mice were given feed containing 1,2-diphenylhydrazine at a lower or higher dose for 78 weeks. The experiments at the lower and higher doses were conducted separately, each dose group having its own control group for males and females. In male and female rats at the lower dose (dietary concentration, 0.008% and 0.004%, respectively), there was an increased incidence of inflammation in several tissues, including the myocardium, lung, and pancreas, relative to the respective control groups. Similarly, in male and female mice at the lower dose (same doses as in rats), there was an increased incidence of inflammation in several tissues, including the lymph nodes and kidney, relative to the respective control groups. [The Working Group noted that in both sexes and species, the results for the higher-dose studies were often inconsistent with those from the lower-dose studies. In addition, the incidence of inflammation was often higher in the controls for the higher dose than in the controls for the lower dose.]

Regarding alterations in cell proliferation, cell death, or nutrient supply, in the abovementioned study by the NCI (NTP, 1978), exposure to 1,2-diphenylhydrazine in male and female rats at the lower dose increased hyperplasia in a few tissues, including the liver, relative to the respective controls. Exposure to 1,2-diphenylhydrazine in male and female mice at the lower dose led to an increase in hyperplasia, notably in the spleen, relative to the respective controls. [The Working Group noted that in both sexes and species, the results for the higher-dose studies were often inconsistent with those from the lower-dose studies. In addition, the incidence of hyperplasia was often higher in the controls for the higher dose than in the controls for the lower dose. Concerning the chronic inflammation and hyperplasia observed in the NCI study (NTP, 1978), the Working Group noted that none of the non-neoplastic lesions in rats or mice appeared to be treatment-related.] Kitchin et al. (1994) reported that rats treated with 1,2-diphenylhydrazine at a dose of 60 or 130 mg/kg bw by gavage had elevated levels of hepatic ornithine decarboxylase activity, indicative of increased cell proliferation.

4.2.3 High-throughput in vitro toxicity screening data evaluation

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 130 was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). 1,2-Diphenylhydrazine was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available. A supplementary table (Annex 2, Supplementary material for Section 4, Mechanistic Evidence, web only; available from: https://publications.iarc.fr/611) provides a summary of the findings (including the assay name, the corresponding key characteristic, the resulting "hit calls" both positive and negative, and any reported caution flags for 1,2-diphenylhydrazine (US EPA, 2021c). The results were generated with the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) (available from: https://gitlab.com/i1650/kc-hits) using the US EPA ToxCast and Tox21 assay data and the curated mapping of key characteristics to assays available at the time of the evaluations performed for the present monograph. Findings and interpretations from these high-throughput assays for 1,2-diphenylhydrazine are discussed below.

After mapping against the key characteristics of carcinogens, the ToxCast/Tox21 database contained 290 assays in which 1,2-diphenylhydrazine was tested. Of these, it was found to be active and without caution flags in 24 assays relevant to the key characteristics of carcinogens [The Working Group noted that the cytotoxic limit for 1,2-diphenylhydrazine is 11.17 μM.]

1,2-Diphenylhydrazine was active in four assays mapped to key characteristic 2 (KC2), "is genotoxic". Two of these assays were conducted in chicken lymphoblasts and the half-maximal activity concentration (AC_{50}) was between 22.52 and 24.58 μM; two other assays were conducted in HepG2 cells and the AC_{50} was 104.2 μM.

1,2-Diphenylhydrazine was active in four assays mapped to KC5, "induces oxidative stress". In HepG2 cells, 1,2-diphenylhydrazine was active in one assay that measures nuclear factor erythroid 2-related factor 2 (NRF2) activity, at an AC_{50} of 32.7 μM, and in one assay that measures the level of phosphorylated H2A histone family, at an AC_{50} of 99.1 μM. 1,2-Diphenylhydrazine was active in one assay that measures the activity of cyclooxygenase in sheep testis, at an AC_{50} of 2.68 μM, and in one assay that measures monooxygenase activity in *E. coli*, at an AC_{50} of 4.26 μM.

The chemical was active in seven assays mapped to KC8, "modulates receptor-mediated effects". In HepG2 cells, 1,2-diphenylhydrazine activated nuclear receptors estrogen receptor α (ERα) and peroxisome proliferator-activated receptor gamma (PPARγ) at an AC_{50} of 60.1 and 77.4 μM, respectively. In addition, 1,2-diphenylhydrazine activated ERα at an AC_{50} of 60.1 μM in the human ovary cell line, VM7, and the thyroid hormone receptor α (THRA) and thyroid hormone receptor β (THRB) at an AC_{50} of 50.6 μM in the rat pituitary gland cell line, GH3. In addition, it was active in three assays that measured changes in the expression of the transcription factors for CYP1A1, CYP1A2, and CYP2B6 in metabolically competent HepaRG liver cells, at an AC_{50} of between 5.08 and 35.7 μM.

Finally, 1,2-diphenylhydrazine was active in nine assays mapped to KC10, "alters cell proliferation, cell death, or nutrient supply". However, only one assay indicated an increase in cell proliferation in HepG2 cells, at an AC_{50} of 112.5 μM. The other eight assays showed a loss of cell viability.

5. Summary of Data Reported

5.1 Exposure characterization

1,2-Diphenylhydrazine was used widely as a chemical precursor for the production of benzidine-based dyes until the late 1970s, after which its use declined significantly due to the phasing out of benzidine-based dyes in many countries. 1,2-Diphenylhydrazine has an additional use as a chemical intermediate in the manufacture of some anti-inflammatory and uricosuric medications, but this application is now also less common owing to discontinued or very limited use of these drugs in human medicine.

1,2-Diphenylhydrazine is occasionally detected in groundwater, surface water, sediment, and waste-water samples. Very few reports and publications were available to assess current and historical occupational and environmental exposure to 1,2-diphenylhydrazine. Because of limited use and relatively rapid degradation in most environmental media, current occupational and environmental exposure to 1,2-diphenylhydrazine is expected to be low.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Treatment with 1,2-diphenylhydrazine caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two species.

1,2-Diphenylhydrazine was administered by oral administration (in the feed) in one study in $B6C3F_1$ mice. In females, 1,2-diphenylhydrazine caused an increase in the incidence of hepatocellular carcinoma and of hepatocellular adenoma or carcinoma (combined).

1,2-Diphenylhydrazine was administered by oral administration (in the feed) in two concurrent studies in Fischer 344 rats. In males, 1,2-diphenylhydrazine caused an increase in the incidence of hepatocellular carcinoma, and of benign and malignant liver tumours (neoplastic nodule or hepatocellular carcinoma, combined) in both studies; and an increase in the incidence of squamous cell carcinoma of the Zymbal gland, squamous cell papilloma or carcinoma (combined) of the ear canal, Zymbal gland or skin of the ear (combined), and benign or malignant (combined) pheochromocytoma of the adrenal gland in one study. In females, 1,2-diphenylhydrazine caused an increase in the incidence of mammary gland adenocarcinoma (not otherwise specified) in one study.

1,2-Diphenylhydrazine was administered by intraperitoneal injection in one study in strain A mice. In males, 1,2-diphenylhydrazine caused an increase in the incidence and multiplicity of pulmonary tumours (not specified as benign or malignant). In females, 1,2-diphenylhydrazine caused an increase in the multiplicity of pulmonary tumours.

5.4 Mechanistic evidence

No data on absorption, distribution, metabolism, and excretion in humans exposed to 1,2-diphenylhydrazine were available to the Working Group. In rodents, two studies demonstrated that 1,2-diphenylhydrazine can be absorbed via multiple routes of exposure and is excreted as parent compound and/or metabolites in the urine. One of these studies reported that aniline, benzidine, and several unidentified metabolites were found in the urine; however, the evidence for the formation of these metabolites is suggestive but inconclusive.

There was no mechanistic evidence available for 1,2-diphenylhydrazine regarding the key characteristics of carcinogens in exposed humans or human cells in vitro. Overall, the

mechanistic evidence regarding the key characteristics of carcinogens ("is genotoxic", "induces oxidative stress", and "alters cell proliferation, cell death, or nutrient supply") is suggestive but incoherent across different experimental systems. 1,2-Diphenylhydrazine was shown in rodents to inhibit testicular DNA synthesis in one study but did not cause hepatic DNA strand breaks in another. The chemical caused chromosome aberration and sister-chromatid exchange in Chinese hamster ovary cells in one study but only in the presence of metabolic activation. 1,2-Diphenylhydrazine was mutagenic in two studies in one strain of bacteria with and without metabolic activation but not in multiple other strains. The chemical gave negative results for mutagenicity in *Drosophila melanogaster*, but positive results for DNA damage and oxidative stress in the presence of copper(II) in an acellular system. For the key characteristic "alters cell proliferation, cell death, or nutrient supply", there was a paucity of available data. In one study in rodents, 1,2-diphenylhydrazine caused an elevation in hepatic ornithine decarboxylase activity, indicative of increased cell proliferation.

1,2-Diphenylhydrazine was found to be mostly without effects in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes in the USA.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of 1,2-diphenylhydrazine.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,2-diphenylhydrazine.

6.3 Mechanistic evidence

There is *limited mechanistic evidence*.

6.4 Overall evaluation

1,2-Diphenylhydrazine is *possibly carcinogenic to humans (Group 2B)*.

6.5 Rationale

The Group 2B evaluation for 1,2-diphenylhydrazine is based on *sufficient evidence* for cancer in experimental animals. The *sufficient evidence* in experimental animals is based on an increased incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two species. The evidence regarding cancer in humans is *inadequate* because no studies were available. The mechanistic evidence was *limited* as the findings regarding key characteristics of carcinogens across experimental systems were suggestive, but incoherent.

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