Addendum to the
Toxicological Profile for 1,2-Diphenylhydrazine

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine
Atlanta, GA 30333

October 2009

CONTENTS

Tables

ADDENDUM for 1,2-Diphenylhydrazine
Supplement to the 1990 Toxicological Profile for 1,2-Diphenylhydrazine

Background Statement

This addendum to the Toxicological Profile for 1,2-Diphenylhydrazine supplements the profile that was released in 1990.

Toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). CERCLA mandates that the Administrator of ATSDR prepare toxicological profiles on substances on the Priority List of Hazardous Substances and that the profiles be revised "no less often than once every three years". CERCLA further states that the Administrator will "establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" [Title 42, Chapter 103, Subchapter I, § 9604 (i)(1)(B)].

The purpose of this addendum is to provide, to the public and other federal, state, and local agencies, a non-peer reviewed supplement of the scientific data that were published in the open peer-reviewed literature since the release of the profile in 1990.

Chapter numbers in this addendum coincide with the Toxicological Profile for 1,2-Diphenylhydrazine (1990). This document should be used in conjunction with the profile. It does not replace it.

2. HEALTH EFFECTS

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

2.2.2 Oral Exposure

2.2.2.7 Genotoxic Effects

The relationships among chemical structure, carcinogenicity, and mutagenicity for 301 chemicals tested by the National Toxicology Program (NTP) was assessed. 1,2-Diphenylhydrazine was identified as one of only 12 that induced tumors of the Zymbal's gland. Virtually all these are mutagenic to Salmonella, and they are found in the rat, but not in the mouse. The authors concluded that Zymbal's gland tumor induction by 1,2-diphenylhydrazine and the others is a unique result of genotoxic carcinogenesis (Ashby and Tennant 1991). It has been noted that a positive Ames test result was found for 1,2-diphenylhydrazine by the NTP (Benigni 1990).

2.2.2.8 Cancer Effects

A total of 116 NTP rodent carcinogens were classified on the basis of the tumors they produce, using two multivariate data analysis methods (cluster and factor analysis to reduce the number of original variables); Benigni then compared the results with Ames-positive and Ames-negative chemicals (Benigni 1990). The recognized tumor sites for 1,2-diphenylhydrazine from the NTP studies include the liver (carcinoma for the male rat, female rat, and female mouse), mammary gland (for the female rat), and Zymbal's gland (for the male rat). The data were transformed into a dissimilarity matrix, and a clustering algorithm was applied in identifying 4 clusters of chemicals. A comparison of clustering with Ames test results revealed that 1,2-diphenylhydrazine and the other chemicals in cluster 4 showed a significant association with Ames test results. Some of those other substances included aniline, 1,3-dichloropropene, 1,4-dioxane, polybrominated biphenyl, and 2,3,7,8-tetrachlorodibenzo-p-dioxin, The sites of tumors found widely in studies of cluster 4 chemicals, but not yet reported for 1,2-diphenylhydrazine and indicating the potential for further study, include the circulatory system and the stomach in male rats, the urinary bladder in female rats, the circulatory system and lung in female mice, and the circulatory system and pituitary gland in male mice.

A literature search was conducted to identify 110 rodent carcinogens. Each carcinogen was administered by gavage at two dose levels to female Sprague-Dawley rats, and the authors studied the effects by using 4 biochemical assays (Kitchin et al. 1992). 1,2-Diphenylhydrazine was on that list due to a positive Ames test and increased structural alerts in a computational toxicology assessment. The results were that 1,2-diphenylhydrazine induced hepatic ornithine decarboxylase activity (ODC) but had no reported effect on the other three measures (hepatic DNA damage by alkaline elution, serum alanine amitotransferase activity, and hepatic cytochrome P-450 content). This finding indicated to the authors that 1,2-diphenylhydrazine does not induce cell proliferation, as was the case with 30 of the tested chemicals.

The mechanism by which 1,2-diphenylhydrazine and azobenzene cause DNA damage that can result in cancer was evaluated (Ohnishi et al. 2000). They radiolabeled DNA fragments from the c-Ha-ras-1 protooncogene and the p53 tumor suppressor gene with ^{32}P. In the presence of Cu(II), 1,2-diphenylhydrazine produced DNA damage that was enhanced by the addition of piperidine. The authors found that 1,2-diphenylhydrazine caused damage at thymidine residues and the modification and liberation of DNA base fragments. In light of these results and the realization that the conversion of 1,2-diphenylhydrazine to azobenzene results in the reduction of oxygen, the authors suggested that copper-induced DNA damage with peroxide generation may be involved in its carcinogenesis.

2.3 TOXICOKINETICS

2.3.3 Metabolism

The stability of 1,2-diphenylhydrazine was studied in human male and female gastric juices of differing pH (Assi et al. 1996). At a pH below 5, a continuous decrease in 1,2-diphenylhydrazine occurred until only 2.5% remained after 3 hours. It was pepsin, and not pepsinogen, that readily bound the 1,2-diphenylhydrazine. At a pH of 5–6, minimal change occurred. The implication was that stomach pH can alter the toxicity of this substance.

The metabolites of 1,2-diphenylhydrazine in male SPF Sprague Dawley rats have been identified (Globig et al. 1996). The liver was homogenized and 1,2-diphenylhydrazine in DMSO was added to produce a concentration of 0.03 mg/ml. Samples were extracted with petroleum ether after 1, 5, 10, 20, 30, and 60 minutes and analyzed by HPLC. The detected metabolites included aniline, azobenzene, 2-amiondiphenylamine, and benzidene. Although only half the 1,2-diphenylhydrazine remained unmetabolized after 1 hour, time-dependent kinetics were unresolvable. In addition, some anticipated metabolites were absent, indicating that intact hepatocytes are required for the production of hydroxylated metabolites.

The metabolites of 1,2-diphenylhydrazine and azobenzene were identified in hepatocyte cell cultures of male SPF Sprague-Dawley rats (GlobigKillguss 1996). These included metabolites already identified in the toxicological profile (aniline, 4-aminophenol, benzidine, 4-hydroxyazobenzene, and azobenzene) as well as others (2-diaminophenylamine, 2,2'-diaminobiphenyl) . The group then studied the impact of 1,2-diphenylhydrazine and azobenzene on metabolism (Globig and Freundt 1996). When one was added, the other was formed, indicating the reversibility of reactions for this pair.

2.4 RELEVANCE TO PUBLIC HEALTH

1,2-Diphenylhydrazine can irritate the skin, eyes, nose, throat, and lungs. It can cause a skin allergy, and if it does, future low exposures can cause itching and skin rash. Short-term high-level exposure can cause headache, nausea, and dizziness, possibly damaging red blood cells. High or repeated exposure can damage the kidney and liver (NDCRT 2009). 1,2-Diphenylhydrazine has been found to cause cancer in animals and it probably causes cancer in humans.

2.6 INTERACTIONS WITH OTHER CHEMICALS

Cu(II) was evaluated and found to enhance the carcinogenicity of 1,2-diphenylhydrazine, especially when treated with pipridine (OhnishiMurata 2000). ^{32}P-5'-end-labeled DNA from the c-Ha-ras-1 protooncogene and the p53 tumor suppressor gene wre exposed to 1,2-diphenylhydrazine in vitro. DNA damage occurred in the presence of Cu(II), frequently at

thymine residues, but hydroxyl radical scavengers did not mediate the damage. Piperidine treatment significantly increased the damage, indicating that 1,2-diphenylhydrazine produced base modification and liberation. This damage was mediated by the presence of catalase and a Cu(I)-specific chelator.

2.9 MECHANISMS OF ACTION

1,2-Diphenylhydrazine has been found to be carcinogenic to rats and mice. The mechanism was studied in an in vitro system by exposing ^{32}P-5'-end-labeled DNA from the c-Ha-ras-1 protooncogene and the p53 tumor suppressor gene to both azobenzene and its metabolite, 1,2-diphenylhydrazine (OhnishiMurata 2000). DNA damage occurred in the presence of 1,2-diphenylhydrazine and Cu(II), but not with azobenzene. The damage occurred frequently at thymine residues, and it was not mediated by hydroxyl radical scavengers. Piperidine treatment significantly increased the damage, indicating that 1,2-diphenylhydrazine produced base modification and liberation. This finding was supported by an increase in formation of 8-oxo-7,8-dihydro-2'deoxyguanosine. The primary active species was considered to be Cu(I)-OOH or another metal-oxygen complex, along with peroxide, since H_2O_2 was reported to have been associated with the autoxidiation of 1,2-diphenylhydrazine to azobenzene. This damage was mediated by the presence of catalase and a Cu(I)-specific chelator.

3. CHEMICAL AND PHYSICAL INFORMATION

3.1 CHEMICAL IDENTITY

Table 3-1. Chemical Identity of 1,2-Diphenylhydrazine

	Value	Reference
Synonyms	N,N'-bianiline	(IPCS 1993)
	1,1'-Hydrazobisbenzene	(BGChemie 1994)
	Hydrazodibenzene	
	Hydrazobenzol	
	Hydrazobenzen	(HSDB 2009)
	1,1'-Hydrazodibenzene	

3.2 CHEMICAL AND PHYSICAL PROPERTIES

Table 3-2. Physical and Chemical Properties of 1,2-Diphenylhydrazine

Property	Value	Reference
Physical state	White to yellow crystals	IPCS, 1993
Melting point	131ºC	Spectrum Laboratories, 2009
Odor threshold	1-10 ppm	Spectrum Laboratories, 2009
Solubility: Organic solvents	Dissolves readily in ether	(Falbe and Regitz, 1990)
Conversion factors	1 mL/m3 (ppm) ≈ 7.53 mg/m^3 at 1013 hPa and 25 ºC	(BGChemie 1994)

4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

4.3 USE

1,2-Diphenylhydrazine is used as an antisludging additive to motor oil, a desuckering agent for tobacco plants, a reductant in the reclamation of rubber, a component of experimental organometallic polymers, an ingredient in photochromic resin compositions, and a component in polymerization reactions. It can be used in the manufacture of hydrogen peroxide. Some 1,2-diphenylhydrazine derivatives are used as flame-retardant agents (Spectrum Laboratories 2009). Several arylhydrazine interactions in small molecule complexes were studied to see how they might react with iron and other substances (Zdilla et al. 2008). They found that a 3 mM solution of benzene completely disproportionates 6 equivalents of 1-2-DPH into aniline and azobenzene. Their effort to shift the chemistry in a different direction was only partially successful. They concluded that 1,2-diarylhydrazines and their derivatives enjoy complicated chemistries that include structural rearrangement and disproportionation and that their interaction with iron is complex and incompletely understood.

5. POTENTIAL FOR HUMAN EXPOSURE

5.3 ENVIRONMENTAL FATE

5.3.2 Transformation and Degradation

5.3.2.1 Air

Analytical data were compiled for samples collected in support of the Clean Air Act amendments of 1990 and the ABIOTIX method was used to provide the abiotic degradation rate constants in the atmosphere (Kelly et al. 1994). It was reported that 1,2-diphenylhydrazine is rapidly removed or transformed from the atmosphere at a rate of <1 day (the time to reduce the concentration to 37% of its original value). The rates were also rapid for some of its metabolites (aniline < 1 day, benzidene <1 day).

5.3.2.2 Water

The impact of solution pH was found to take a pH of 3 or lower to produce a rearrangement of 1,2 diphenylhydrazine (AssiGlobig 1996). Samples were stored at room temperature, adjusted to pH values ranging from 1 to 7, held for up to 5 hours, and then analyzed by use of HPLC to identify the extent of change and the conversion products. The result at pH 1 was an almost complete transformation to 4-aminophenol and its polymers. At pH 2, a continuous buildup occurred of decomposition products benzidene, 3 aminophenol, and 2,2'-diaminobiphenyl. At pH 3, only benzidene increased continuously, while at pH 4–7, no noticeable changes occurred. Two of the metabolites found at pH2 (3 aminophenol and 2,2'-diaminobiphenyl) are not among those shown on the metabolite scheme in Figure 2-2 of the Toxicological Profile for 1,2-diphenylhydrazine, indicating that environmental decomposition can produce a wider range of metabolites.

Aniline, a metabolite of 1,2-diphenylhydrazine, was found it to decompose by photolysis in the atmosphere (with a 2-day half-life) and in water (with summer and winter half-lives of 36 and 125 hours) (Diment et al. 1993). These times were significantly reduced by the presence of hydroxyl radicals in air and humic acids, some algal species, and other microorganisms that may be present in soil, water, and sewage. The findings suggest that specific environmental

conditions can significantly impact assessments of 1,2-diphenylhydrazine in the environment that are based on the presence of its metabolites.

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.4 Other Environmental Media

1,2-Diphenylhydrazine is present (along with PCBs, and a wide range of other organic contaminants) in coal refuse and in corn, cabbages, and carrots grown in soil amended with it (Webber et al. 1994). Coal refuse is the material separated from coal during the cleaning process, and it includes rock pyrites, slate, shale, mill tailings, clay, and other substances. The study focused on PCBs and did not report the concentrations of 1,2-diphenylhydrazine.

6. ANALYTICAL METHODS

At the time the profile was published (ATSDR 1990), there were no adequate methods for analyzing 1,2-diphenylhydrazine in biological materials, partially due to its rate of metabolism and degradation relative to the length of the sample preparation and analysis process. Although there were sufficient methods for environmental sample analysis, there were no adequate methods for sample collection, preservation, and preparation (EPA 1987a). Also, the analysis was virtually meaningless, since the relative levels in a sample at times of collection and analysis could not be related (Riggin and Howard 1982). Some of these obstacles have since been overcome.

6.2 ENVIRONMENTAL SAMPLES

Some of the previous difficulties with environmental sample analysis have been resolved (Khalil et al. 1999). They compared a single-step acidic extraction process with a 2-step basic and acidic extraction procedure used in EPA Method 625 for over 60 substances. Their method was found to be quicker and to give better results, and EPA Region V reportedly authorized using their method as a site-specific alternative for analyzing industrial wastewater samples. This alternative method used serial extractions of 1,2-diphenylhydrazine with low-pH methylene chloride, passed

the extractant through anhydrous sodium sulfate, concentrated the liquid, and injected it into a GC/MS equipped with a 30-m narrow-bore fused-silica column. The method detection limit for 1,2 diphenylhydrazine was measured at 0.9 µg/L in reagent water and 0.5 µg/L in field samples, compared with no detectability for the EPA 625 method.

Four methods for extracting a range of substances from soil were compared and it was found that all produced excellent recoveries (Lopez-Avila et al. 1996). From lowest to highest, these methods included (along with the % recovery and standard deviation) supercritical fluid extraction with carbon dioxide with 10% methanol (94%, RSD 2.5%), Soxhlet extraction with hexane-acetone (104%, RSD 16%), microwave-assisted extraction with hexane-acetone (113%, RSD 1.1%), and sonication extraction with methylene chloride-acetone (116%, RSD 2.5%). The microwave-assisted extraction method demonstrated high potential for producing reliable samples at the time of analysis.

A method was developed to analyze for trace levels of 1,2 diphenylhydrazine and other substances in a pharmaceutical process stream (Bishop and Mitra 2005). Although tramp substances may be present during pharmaceutical manufacturing at low levels, those levels could be of toxicological importance, making it important to obtain real-time measurements during, rather than after, the manufacturing process. The method used on-line membrane pervaporation (the selective transport of volatile organics across a membrane, in this case Teflon and a sulfonic acid polymer) and the direct injection of the sample stream into an HPLC reverse-phase analytical column. Although the percent recovery was low due to the affinity of 1,2-diphenylhydrazine for the sulfonic membrane, the study demonstrated the feasibility of conducting the real-time analysis of 1,2 diphenylhydrazine in industrial liquid streams, and it indicated the need for studies of other membrane materials. The authors recommended conducting studies on other membrane materials in order to enhance the recovery of 1,2-diphenylhydrazine.

Radioactive waste water was analyzed by using a modified version of EPA method 8270 that protected the operator from radiation exposure (Tomkins et al. 1990). However, it was found that

1,2-diphenylhydrazine was not detectable by this method at the concentrations present in the liquid.

6.3 ADEQUACY OF THE DATABASE

6.3.1 Identification and Data Needs

The sampling of trace levels of 1,2-diphenylhydrazine using a pervaporation technique suffered from low recovery due to the interation with the sufonic acid residues in the membrane (Bishop and Mitra 2005). Identification of a membrane better suited for high recoveries could improve detectability of 1,2-diphenylhydrazine.

7. REGULATIONS AND ADVISORIES

The National Toxicology Program (NTP 2002) has identified 1,2-diphenylhydrazine as reasonably anticipated to be a human carcinogen on the basis of sufficient evidence of carcinogenicity in experimental animals. Also, it is classified as probable human carcinogen by the US EPA (EPA 2009). Table 7-1 identifies the regulations and advisories applicable to this substance.

Table 7-1. Regulations and Advisories Applicable to 1,2-Diphenylhydrazine

Agency	Description	Value	Reference
	National		
Regulations:			
a. Nonspecific media:			
EPA OERR	Reportable quantity	10 pounds	EPA 2009 40CRF 302.4
Guidelines:			
b. Water:			
EPA	Water quality criteria		EPA, 33 U.S.C.
	-Fish/shellfish and water consumption	0.036 µg/L	§1251 et seq.
	-Fish/shellfish consumption	0.20 µg/L	NTP 2005
	State		
b. Water:	Drinking Water Guidelines		
AZ		0.05 µg/l	HSDB 2009
FL		10 µg/l	HSDB 2009
NH		0.05 µg/l	HSDB 2009

CFR = Code of Federal Regulations
EPA = Environmental Protection Agency
HSDB = Hazardous Substances Data Bank
NTP = National Toxicology Program
OERR = Office of Emergency and Remedial Response

8. REFERENCES

Ashby J, Tennant RW. 1991. Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. Mutat Res 257(3):229–306.

Assi A, Globig S, Koelbel C, et al. 1996. Ph-dependent reaction types of hydrazobenzene in gastric juice and hydrous solution. 37th Spring Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology, Mainz, Germany, March 12-14, 1996 Naunyn-Schmiedeberg's Archives of Pharmacology 353(4 SUPPL.):R112.

Benigni R. 1990. Rodent tumor profiles, Salmonella mutagenicity and risk assessment. Mutat Res 244(1):77–91.

BGChemie. 1994. Toxicological Evaluations: BG-No. 19 Hydrazobenzene: Berufsgenossenschaft Chemie. Frankfurt.

Bishop EJ, Mitra S. 2005. On-line membrane preconcentration for continuous monitoring of trace pharmaceuticals. J Pharm Biomed Anal 37(1):81–86.

Diment J, Dobson S, Nielsen IR. 1993. Environmental hazard assessment: aniline. Toxic Substances Division Department of the Environment, London 15(1993).

EPA. Integrated Risk Management System: 1,2-Diphenylhydrazine. Environmental Protection Agency. Available from: http://www.epa.gov/iris/subst/0049.htm.

Globig S, Freundt KJ. 1996. Metabolism of hydrazobenzene in rat liver homogenate. Autumn Meeting of the Deutsche Gesellschaft Fuer Experimentelle Und Klinische Pharmakologie Und Toxikologie (German Society for Experimental and Clinical Pharmacology and Toxicology), Dresden, Germany, September 10-12, 1996 Naunyn-Schmiedeberg's Archives of Pharmacology 354(4 SUPPL. 1):R28.

Globig S, Killguss M, and Freundt K. 1996. Metabolism of hydraobenzene and azobenzene in rat hepatocytes in primary culture. Autumn Meeting of the Deutsche Gesellschaft Fuer Experimentelle Und Klinische Pharmakologie Und Toxikologie (German Society for Experimental and Clinical Pharmacology and Toxicology), Dresden, Germany, September 10-12, 1996 Naunyn-Schmiedeberg's Archives of Pharmacology 354(4 SUPPL. 1):R419.

HSDB. 2009. Hazardous Substances Data Bank. National Library of Medicine, National Toxicology Information Program, Bethesda, MD.

IPCS. 1993. International Chemical Safety Card for 1,2-Diphenylhydrazine. In: International Programme for Chemical Safety WHO, editor. Geneva.

Kelly TJ, Mukund R, Spicer CW, et al. 1994. Concentrations and transformations of hazardous air pollutants. Environmental Science & Technology 28(8):378A–387A.

Khalil MS, Kelada N, Sawyer B, et al. 1999. Comparison of one-step acidic extraction versus two-step basic and acidic extraction procedures for semivolatile analysis of wastewater. Water Environ Res 71(3):348–352.

Kitchin KT, Brown JL, Kulkarni AP. 1992. Predictive assay for rodent carcinogenicity using in vivo biochemical parameters: operational characteristics and complementarity. Mutat Res 266(2):253–272.

Lopez-Avila V, Young R, Teplitsky N. 1996. Microwave-assisted extraction as an alternative to soxhlet, sonication, and supercritical fluid extraction. J AOAC Int 79(1):142–156.

NDCRT. 2009. Chemical Fact Sheet for 1,2-Diphenylhydrazine. The Metropolitan Emergency Response & Logistical Information Network.

NTP. 2002. Hydrazobenzene. Rep Carcinog 10:139–140.

Ohnishi S, Murata M, Oikawa S, et al. 2000. Copper-dependent DNA damage induced by hydrazobenzene, an azobenzene metabolite. Free Radic Res 32(6):469–478.

Tomkins BA, Caton JEJ, Fleming GS, et al. 1990. Determination of regulatory organic compounds in radioactive waste samples: Semivolatile organics in aqueous liquids. Anal Chem 62(3):253–257.

Webber MD, Pietz RI, Granato TC, et al. 1994. Plant uptake of PCBs and other organic contaminants from sludge-treated coal refuse. Journal of Environmental Quality 23(5):1019–26.

Zdilla MJ, Verma AK, Lee SC. 2008. Reactivity of a sterically hindered Fe(II) thiolate dimer with amines and hydrazines. Inorg Chem 47(23):11382–11390.