Toxicological Profile for 3,3'-Dichlorobenzidine

June 2022

U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

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DISCLAIMER

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance due to associated acute, intermediate, and chronic exposures;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, intermediate, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staffs of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

VERSION HISTORY

Date	Description
June 2022	Final toxicological profile released
July 2021	Draft for public comment toxicological profile released
May 2020	Addendum to the toxicological profile released
December 1998	Final toxicological profile released
October 1989	Final toxicological profile released

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These experts collectively have knowledge of toxicology, chemistry, and/or health effects. All reviewers were selected in conformity with Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

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CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

1.1 OVERVIEW AND U.S. EXPOSURES

3,3'-Dichlorobenzidine is a synthetic chlorinated primary aromatic amine structurally similar to benzidine, which is designated as a human carcinogen. It is used in the production of azo dyes and pigments in the textile, inks, plastics, rubber, and leather industries. In the United States, manufacturing use has significantly decreased since its classification as a possible human carcinogen, although its use in manufacturing processes abroad continues.

Among the general population, people living near production facilities or hazardous waste sites are at risk of 3,3'-dichlorobenzidine exposure through industrial wastewater effluents or groundwater contamination. 3,3'-Dichlorobenzidine has been detected over 6 km away from its source, and in wastewater effluent, can travel long distances when bound to sediment. In wastewater effluent, 3,3'-dichlorobenzidine has been measured at concentrations ranging from non-detectable to 654 μg/L with an estimated median concentration <10 μg/L. Trace amount of 3,3'-dichlorobenzidine has also been detected in cosmetics that use colorants containing azo dyes in concentrations of 0.16–1.70 mg/kg; the highest concentration was observed in cosmetic skin care facial masks. Laboratory studies have observed that the photodegradation of some yellow and orange pigments used in permanent skin tattoos can yield 3,3'-dichlorobenzidine. This suggests a possible dermal exposure route, although this process has yet to be observed in humans. Due to its use as a pigment in paint, exposure to 3,3'-dichlorobenzidine could occur through ingestion of paint chip debris; this is of particular concern for children. 3,3'-Dichlorobenzidine has also been found in trace amounts in some cosmetics, skin care, and personal hygiene products in China.

Manufacturing workers of processes using or producing 3,3'-dichlorobenzidine are most likely to be exposed by inhalation or dermal contact. In occupational settings, 3,3'-dichlorobenzidine has been reported at air concentrations ranging from ≤0.6 to 2.5 μg/m^3. Dermal exposure occurs when workers handle the chemical; however, exposures have not been quantified.

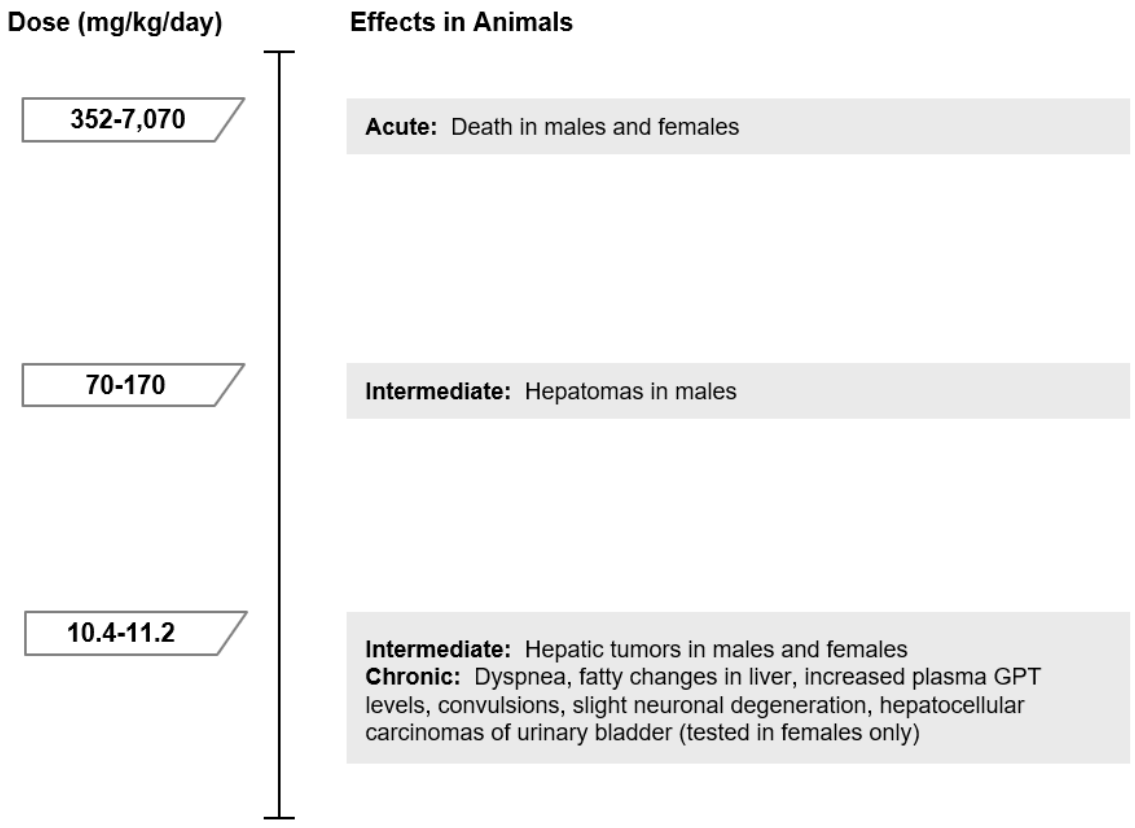
3,3’-Dichlorobenzidine and 3,3’-dichlorobenzidine metabolites are in excreted in urine; therefore, urinary levels of 3,3’-dichlorobenzidine and its metabolites are used as biomarkers of exposure. In addition, 3,3’-dichlorobenzidine metabolites can form adducts with hemoglobin and DNA, and the adducts are considered to be early biological effects of 3,3-dichlorobenzidine. Detection of these adducts can be used as both biomarkers of exposure and biomarkers of effect. Monitoring of hemoglobin and DNA adducts

combined with measuring urinary 3,3'-dichlorobenzidine and metabolite levels are effective tools for biological monitoring in humans. There are no baseline or "normal" human values for either urine or hemoglobin adduct levels for 3,3'-dichlorobenzidine. The lowest limit of detection for 3,3'-dichlorobenzidine in urine is 1.6 µg/L; and the lowest limit of detection for hemoglobin adducts is <0.1 ng/g (see Table 5-1).

1.2 SUMMARY OF HEALTH EFFECTS

The adverse health effects of 3,3'-dichlorobenzidine have been evaluated in a small number of epidemiology and animal studies. The available observational epidemiology studies primarily examined cancer as an endpoint. Most of the health effects data come from oral exposure studies in animals which evaluated the following endpoints: cancer, genotoxicity, neurological, endocrine, ocular, dermal, renal, hepatic, respiratory, body weight, and death. The lowest-observed-adverse-effect levels (LOAELs) seen in studies of animals orally exposed to 3,3'-dichlorobenzidine are presented in Figure 1-1. Exposure durations are defined as follows: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

Figure 1-1. Health Effects Found in Animals Following Oral Exposure to 3,3'-Dichlorobenzidine



1. RELEVANCE TO PUBLIC HEALTH

The most sensitive effects appear to be cancer and genotoxicity. Cancer studies were evaluated in both humans and animals. The U.S. Department of Health and Human Services classifies 3,3'-dichlorobenzidine as reasonably anticipated to be a human carcinogen (NTP 2016). The U.S. Environmental Protection Agency (EPA) classifies it as B2; probable human carcinogen (IRIS 2006). The International Agency for Research on Cancer (IARC) classifies 3,3'-dichlorobenzidine as possibly carcinogenic to humans (Group 2B) (IARC 1987).

Respiratory Effects. Limited animal findings include slight-to-moderate pulmonary congestion and pulmonary abscesses in rats following inhalation exposure, and dyspnea in one dog following oral exposure to 3,3'-dichlorobenzidine.

Hepatic Effects. Limited animal evidence suggests that chronic-duration oral exposure to 3,3'-dichlorobenzidine can result in mild-to-moderate liver injury.

***Dermal Effects*.** Dermatitis was cited as a health problem encountered by workers in contact with the free base of 3,3'-dichlorobenzidine in a manufacturing plant of the chemical (Gerarde and Gerarde 1974).

Neurological Effects. Convulsions and slight neuronal degeneration were seen in a single dog given an oral dose of 3,3'-dichlorobenzidine (Stula et al. 1978).

Cancer. The seven human studies identified involved occupationally exposed populations. The findings of the studies are mixed, and they are not of high quality. Three of the six studies found no association between exposure and bladder cancer incidence (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975). However, these three studies had limited power to detect an exposure-related effect due to small sample size, short follow-up time, no control group, or co-exposure to other chemicals. Two of the six studies reported an increase in bladder tumors in workers exposed to either benzidine-based azo dyes (Myslak et al. 1991) or arylamines (Ouellet-Hellstrom and Rench 1996). While the workers were exposed to 3,3'-dichlorobenzidine, they were exposed to other chemicals linked to bladder cancer. Rosenman and Reilly (2004), identified one case of bladder cancer among workers exposed only to 3,3'-dichlorobenzidine in a chemical manufacturing plant. Among these same workers, an increased risk for lymphohematopoietic cancer was identified (standardized mortality ratio [SMR] 6.62; 95% confidence interval [CI] 1.37–19.36). A limitation of this study is the lack of quantitative exposure data for the workers as only the number of years worked was provided. A follow-up to this study did not find associations between 3,3'-dichlorobenzidine and any cancer type (Millerick-May et al. 2021). The

evidence for carcinogenicity of 3,3'-dichlorobenzidine comes from studies on multiple animals and models. 3,3'-Dichlorobenzidine has been found to be carcinogenic in rats, mice, dogs, and equivocal in hamsters (Osanai 1976; Pliss 1959; Stula et al. 1975, 1978).

Genotoxic Effects. There is evidence of the genotoxicity of 3,3'-dichlorobenzidine, both *in vitro* and *in vivo* assays. Results of *in vitro* assays showed that 3,3'-dichlorobenzidine was mutagenic, increased the frequency of sister chromatid exchanges, damaged deoxyribonucleic acid (DNA), and demonstrated unscheduled DNA synthesis (Bratcher and Sikka 1982; Chen et al. 2003, 2014; Chung et al. 2000; Claxton et al. 2001; Hering et al. 2018;Imaoka et al. 1997; Lazear et al. 1979; Makena and Chung 2007; Savard and Josephy 1986; Shiraishi 1986; Vithayathil et al. 1983; Wang et al. 2005).

Results of *in vivo* assays in rats and mice showed micronuclei induction, unscheduled DNA synthesis, DNA damage, and DNA binding (Ashby and Mohammed 1988; Cihak and Vontorkova 1987; Ghosal and Iba 1990; Morita et al. 1997; Sasaki et al. 1999).

1.3 MINIMAL RISK LEVELS (MRLs)

The inhalation database was considered inadequate for the derivation of an MRL. The most sensitive targets following oral exposure to 3,3'-dichlorobenzidine are presented in Figure 1-2. The oral database was limited, and data were considered inadequate for the derivation of an MRL. MRL information for 3,3'-dichlorobenzidine is summarized in Table 1-1.

Figure 1-2. Summary of Sensitive Targets of 3,3'-Dichlorobenzidine – Oral

Hepatic, respiratory, and neurological effects are the most sensitive noncancer targets of 3,3'-dichlorobenzidine oral exposure. Cancer is the most sensitive effect.
Numbers in circles are the lowest LOAELs among health effects in animals.

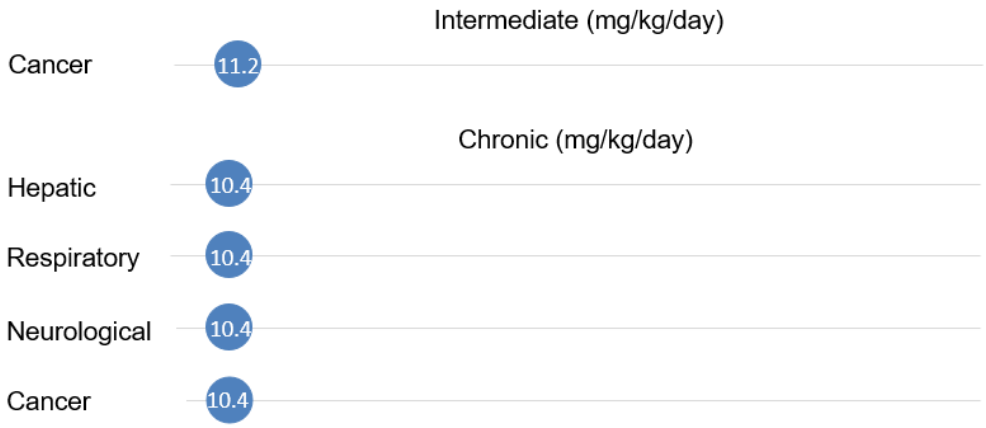


Table 1-1. Minimal Risk Levels (MRLs) for 3,3'-Dichlorobenzidine[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty factor	Reference
Inhalation exposure (ppm)					
Acute		Insufficient data for MRL derivation			
Intermediate		Insufficient data for MRL derivation			
Chronic		Insufficient data for MRL derivation			
Oral exposure (mg/kg/day)					
Acute		Insufficient data for MRL derivation			
Intermediate		Insufficient data for MRL derivation			
Chronic		Insufficient data for MRL derivation			

[a]See Appendix A for additional information.

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 3,3'-dichlorobenzidine. 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.

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 3,3'-dichlorobenzidine, but may not be inclusive of the entire body of literature. Summaries of the human observational studies are presented in Table 2-1.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-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 (SLOAELs) 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 3,3'-dichlorobenzidine are indicated in Table 2-2 and Figure 2-2.

A User's Guide has been provided at the end of this profile (see Appendix C). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

The health effects of 3,3'-dichlorobenzidine have been evaluated in epidemiology and animal studies. As illustrated in Figure 2-1, among the limited number of studies, most examined oral exposure to 3,3'-dichlorobenzidine. The most examined endpoint was cancer. Fifteen studies evaluated toxicity, and these studies examined a limited number of endpoints (genotoxicity, neurological, endocrine, ocular, dermal, renal, hepatic, respiratory, body weight, and death). The small number of available observational epidemiology studies on 3,3'-dichlorobenzidine exposure primarily examined the cancer endpoint. One review of occupational exposures to 3,3'-dichlorobenzidine (Gerarde and Gerarde 1974) stated that exposed workers reported respiratory and neurological symptoms to their company clinic; however, there was insufficient evidence to attribute these effects to exposure. Therefore, these symptoms are not included in Chapter 2.

The human and animal studies suggest the following targets of 3,3'-dichlorobenzidine toxicity:

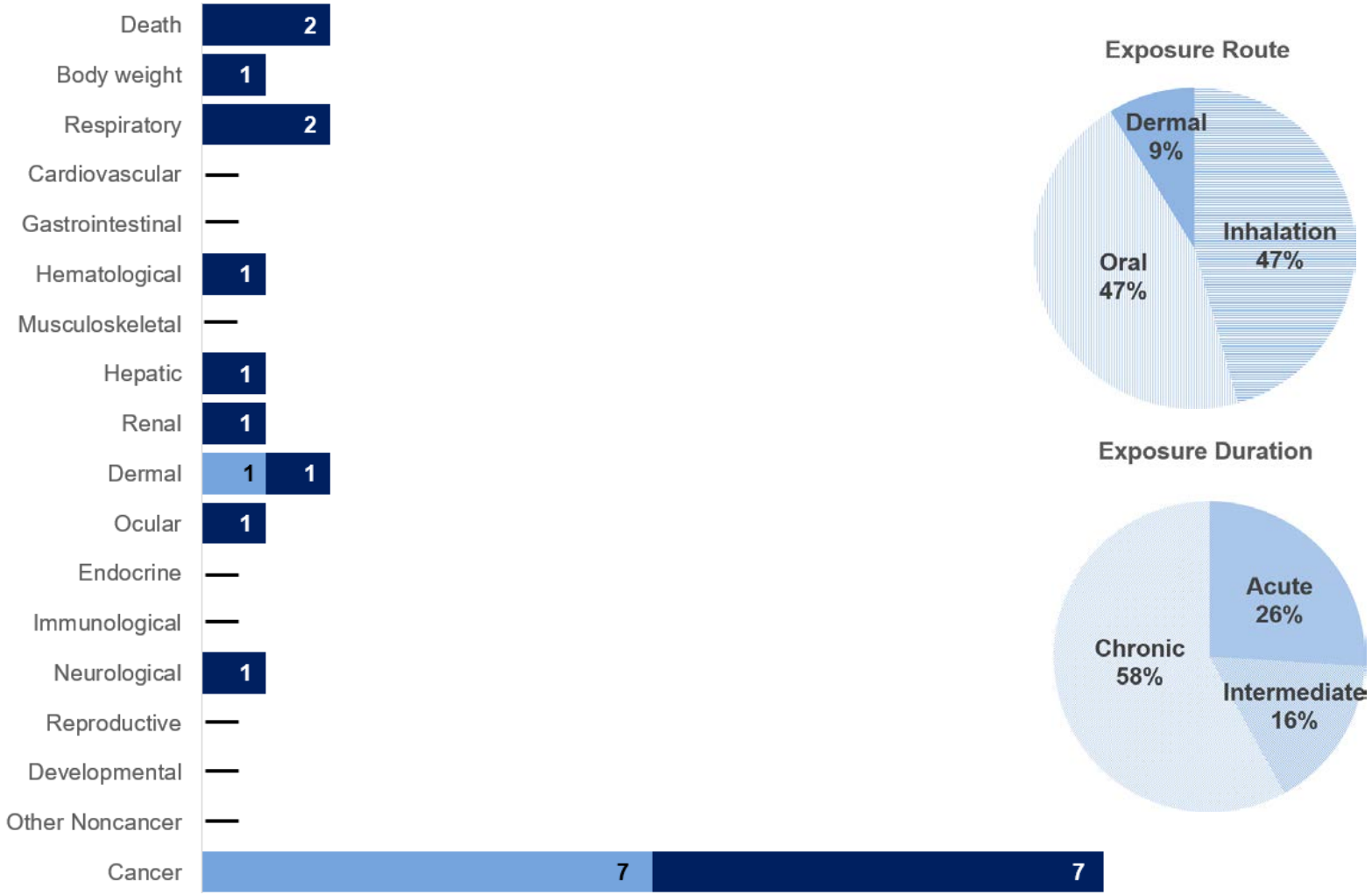
- **Cancer.** Human evidence for cancer is limited to epidemiological studies in occupationally exposed populations. Bladder tumors and increased risk for lymphohematopoietic cancer were identified. Most studies found no association between exposure and bladder cancer incidence. The epidemiological data are limited by sample size, lack of participant follow-up, limited to no exposure data, and co-exposure to other carcinogens. Several organs in animals were impacted by cancer and tumors were found in multiple species.

- **Other endpoints.** Genotoxicity, hepatic, respiratory, and neurological effects have been reported in a limited number of studies, primarily through oral exposure in laboratory animals.

Figure 2-1. Overview of the Number of Studies Examining 3,3'-Dichlorobenzidine Health Effects*

Most studies examined cancerous, hematological, and respiratory effects of 3,3'-dichlorobenzidine.

Fewer studies evaluated **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 16 studies (including those finding no effects) have examined toxicity; some studies examined multiple endpoints

Table 2-1. Health Effects in Humans Exposed to 3,3'-Dichlorobenzidine

Reference and population	Exposure	Outcomes
Gadian 1975 Retrospective study of 59 male workers in dyestuff plant, 35 of whom had exposure to 3,3’-dichlorobenzidine alone Control: 14 male workers, exposed to 3,3'-dichlorobenzidine and other mixed benzidine	Only handled 3,3’-dichlorobenzidine; exposed from 1953 to 1973 for >6 months Exposure range: 975–3,460 total working hours Exposure level: not specified	No bladder cancer found in the group with only 3,3’-dichlorobenzidine exposure.
Gerarde and Gerarde 1974 Retrospective study of 207 (male) workers in a dyestuff plant Control: Not specified	Exposed up to 35 years Exposure level: not specified (study measured exposure in years, not ppm)	The study authors concluded that there is not an increased risk of bladder cancer in the study population; however, reviewers point out several flaws in the study.
MacIntyre 1975 Retrospective study of 225 male and female workers (20–69 years old) exposed to dry and semidry 3,3'-dichlorobenzidine base and hydrochloride Control: not specified	Exposed for an average of <16 years, ranging from <5 to 30 years Exposure levels: not specified	The study concluded that no cases of bladder cancer were identified in exposed workers.
Millerick-May et al. 2021 Follow-up to the retrospective study by Rosenman and Reilly (2004), including 488 male workers Control: U.S. general population rates for cancer mortality used to generate SMR values; Michigan cancer rates for cancer incidence used to generate SIR values	Exposed to benzidine and/or 3,3’-dichlorobenzidine; 227 of 488 workers had exposure to 3,3’-dichlorobenzidine alone between 1960 and 1977 Exposure duration: 0.5–15.75 years (Rosenman and Reilly 2004) Exposure level: information not available	Among workers exposed to 3,3’-dichloro-benzidine, 2 cases of bladder cancer were observed with an SMR of 2.90 (95% CI: 0.07–16.15) and an SIR of 0.89 (95% CI: 0.11–3.23). Among deceased workers exposed to benzidine and/or 3,3’-dichlorobenzidine, four cases of bladder cancer with an SMR 4.10 (95% CI: 1.12–10.50) and SIR for bladder cancer: 3.11 (95% CI: 1.97–4.67).

Table 2-1. Health Effects in Humans Exposed to 3,3'-Dichlorobenzidine

Reference and population	Exposure	Outcomes
Myslak et al. 1991 Case-control study of 403 painters (male) treated between 1984 and 1987 for bladder carcinoma (n=290) and bladder papilloma (n=113) Control: 462 with prostate adenoma (n=345) or prostate hyperplasia (n=81)	Mean duration of employment: 29 years Employment range: 2–48 years; exposed to benzidine-like compounds, including 3,3’-dichlorobenzidine through painting Exposure level: not specified	290 bladder carcinoma diagnoses (21 were painters; 8 controls who were also painters). Relative risk of painters associated with bladder tumors was 2.76 95% CI: 1.21–6.28. Cannot attribute increased risk solely to 3,3’-dichlorobenzidine exposure.
Ouellet-Hellstrom and Rench 1996 Retrospective study of 700 workers (male and female) at a chemical plant Control: Cancer incidence rates from State of Connecticut were used to generate SIR values	Exposure to arylamines, including 3,3'-dichlorobenzidine between 1965 and 1989 Average duration: 4.5 years (males) and 3.1 years (females) Range among workers with bladder cancer, 2.3–20.5 years Exposure level: scoring system of 0–5 based on intensity and frequency of exposure to arylamines (3,3’-dichlorobenzidine, o-dianisidine, o-tolidine, o-toluidine, o-chloroaniline)	Significant increase risk for bladder cancer to workers (SIR 8.3, 95% CI 3.3–17). Cannot attribute increased risk solely to 3,3'-dichlorobenzidine exposure.

Table 2-1. Health Effects in Humans Exposed to 3,3'-Dichlorobenzidine

Reference and population	Exposure	Outcomes
Rosenman and Reilly 2004 Retrospective study of 538 workers (males, 36–62 years old) in a chemical plant Control: U.S. general population cancer mortality rates used to generate SMR values; Michigan cancer incidence rates used to generate SIR values	Exposed to benzidine and/or 3,3’-dichlorobenzidine; 202 of 538 workers had exposure to 3,3’-dichlorobenzidine alone between 1960 and 1972 Exposure duration: 0.5 to 15.75 years Exposure level: information not available	Among deceased workers exposed to benzidine and/or 3,3’-dichlorobenzidine, three cases of bladder cancer with a SMR 8.34, 95% CI: 1.72–24.38. SIR for bladder cancer: 6.85, 95% CI 4.3–10.4. Among same group, six cases of lymphohematopoietic cancer with an SMR 2.84. (95% CI: 1.04–6.18). SMR 6.62; 95% CI: 1.37–19.36 for lymphohematopoietic cancer among workers exposed to 3,3’-dichlorobenzidine only.

CI = confidence interval; SIR = standardized incidence ratio; SMR = standardized mortality ratio

Table 2-2. Levels of Significant Exposure to 3,3'-Dichlorobenzidine – 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)	Effects
ACUTE EXPOSURE									
Gerarde and Gerarde 1974									
1	Rat (albino) NS	Once (GO)		LE	Death			7,070	LD_{50}
Gerarde and Gerarde 1974									
2	Rat (Sprague-Dawley) NS	Once (GO)		LE	Death			3,820	LD_{50}
Gaines and Nelson (1977) as cited in EPA 1980a									
3	Mouse NS	Once	Not specified	LE	Death			488 F	Death
					Death			676 M	Death
Gaines and Nelson (1977) as cited in EPA 1980a									
4	Mouse NS	Once/day 1 week	Not specified	LE	Death			352 F	Death
					Death			386 M	Death
INTERMEDIATE EXPOSURE									
Osanai 1976									
5	Mouse (ICR) 26–39M	6 or 12 months (F)	0, 170	GN	Cancer			170 M	Hepatomas in 8/8 at 6 months and in 18/18 at 12 months

Table 2-2. Levels of Significant Exposure to 3,3'-Dichlorobenzidine – 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)	Effects
Pliss 1959									
6	Mouse (Strain D) 51M, 22F	10 months (F)	11.2–11.9	GN HP LE	Cancer			11.2	Hepatic tumors in 4/18
CHRONIC EXPOSURE									
Pliss 1959									
7	Rat (Rappolovskii) 35M, 15F	6 days/week 12 months (F)	0, 120	GN HP LE	Cancer			120	Carcinoma of Zymbal gland, skin, mammary gland, ileum, bladder, hematopoietic, connective tissue, salivary gland, liver, thyroid
Stula et al. 1975									
8	Rat (Sprague-Dawley) 50M, 50F	16 months *ad libitum* (F)	0, 70 (M), 80 (F)	GN HP	Cancer			80 F 70 M	Malignant mammary gland adenocarcinomas in 26/44 females; malignant mammary gland adeno-carcinomas in 7/44 males; Zymbal gland squamous cell carcinomas in 8/44 males; granulocytic leukemia in 9/44 males
Stula et al. 1978									
9	Dog (Beagle) 6F	3 times week/ 6 weeks + 5 times week up to 7.1 years (C)	0, 10.4	GN HP LE BW UR HE BC CS	Cancer			10.4 F	Hepatocellular carcinomas in 4/6, papillary transitional cell carcinomas of urinary bladder in 5/6
					Bd wt	10.4 F			
					Resp		10.4 F		Dyspnea in 1/6
					Hemato	10.4 F			

Table 2-2. Levels of Significant Exposure to 3,3'-Dichlorobenzidine – 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)	Effects
					Hepatic		10.4 F		Increased plasma ALT levels; fatty changes in liver in 1/6
					Renal	10.4 F			
					Neuro			10.4 F	Convulsions and slight neuronal degeneration in 1/6 dogs

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer are not indicated in Figure 2-2. Where such differences exist, only the levels of effects for the most sensitive gender are presented.

ALT = alanine aminotransferase; BC = blood chemistry; Bd wt or BW = body weight; (C) = capsule; CS = clinical signs; (F) = feed; F = female(s); GN = gross necropsy; (GO) = gavage in oil vehicle; HE = hematology; Hemato = hematological; HP = histopathological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; LD50 = lethal dose, 50% kill; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; UR = urinalysis

Figure 2-2. Levels of Significant Exposure to 3,3'-Dichlorobenzidine – Oral

Acute (≤14 days)

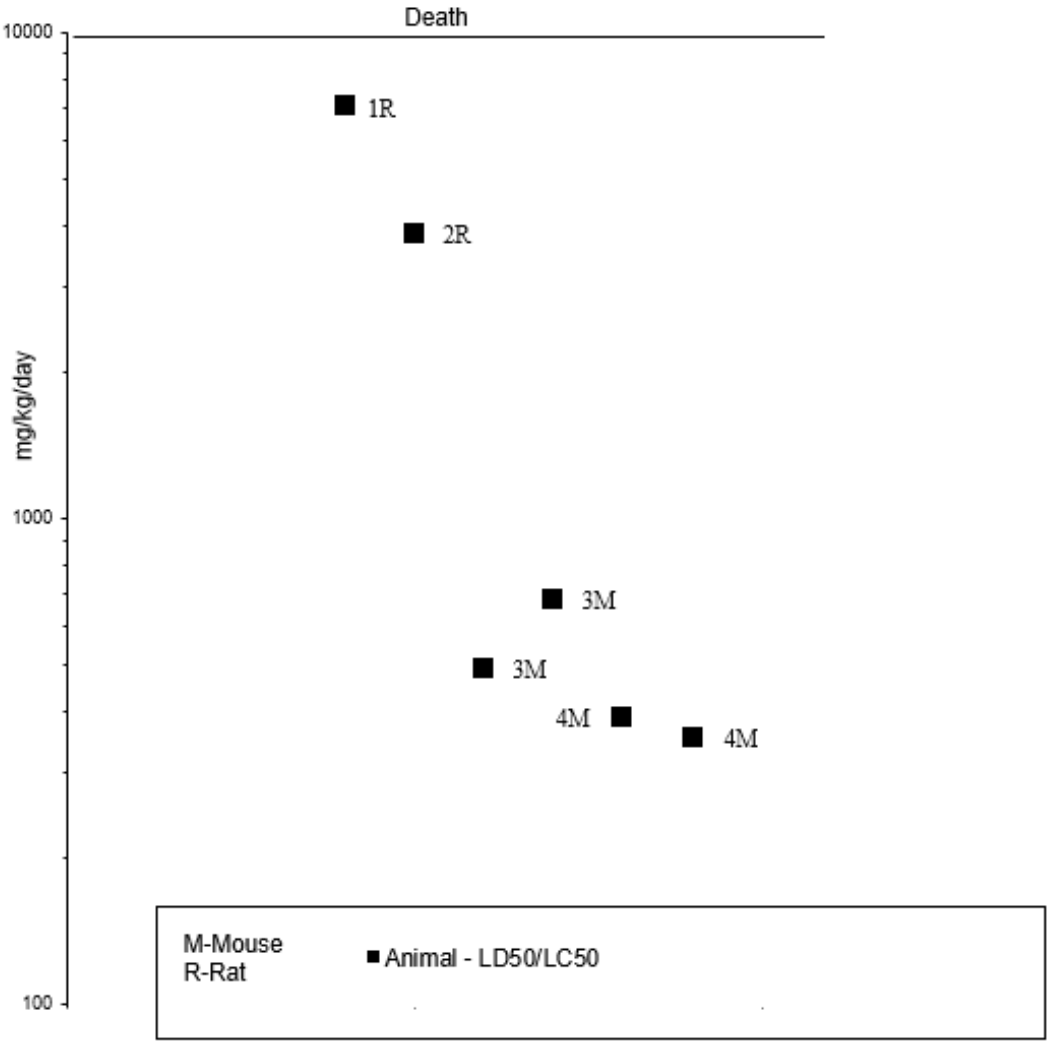


Figure 2-2. Levels of Significant Exposure to 3,3'-Dichlorobenzidine – Oral

Intermediate (15-364 days)

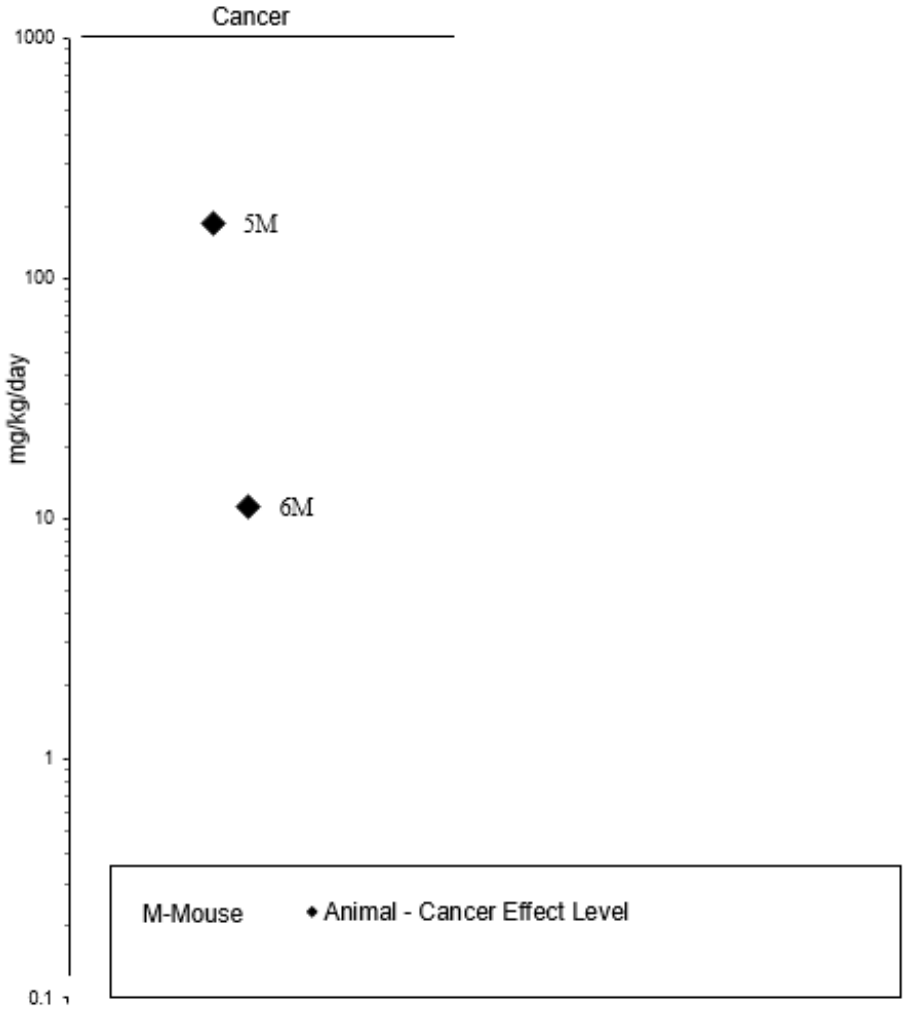
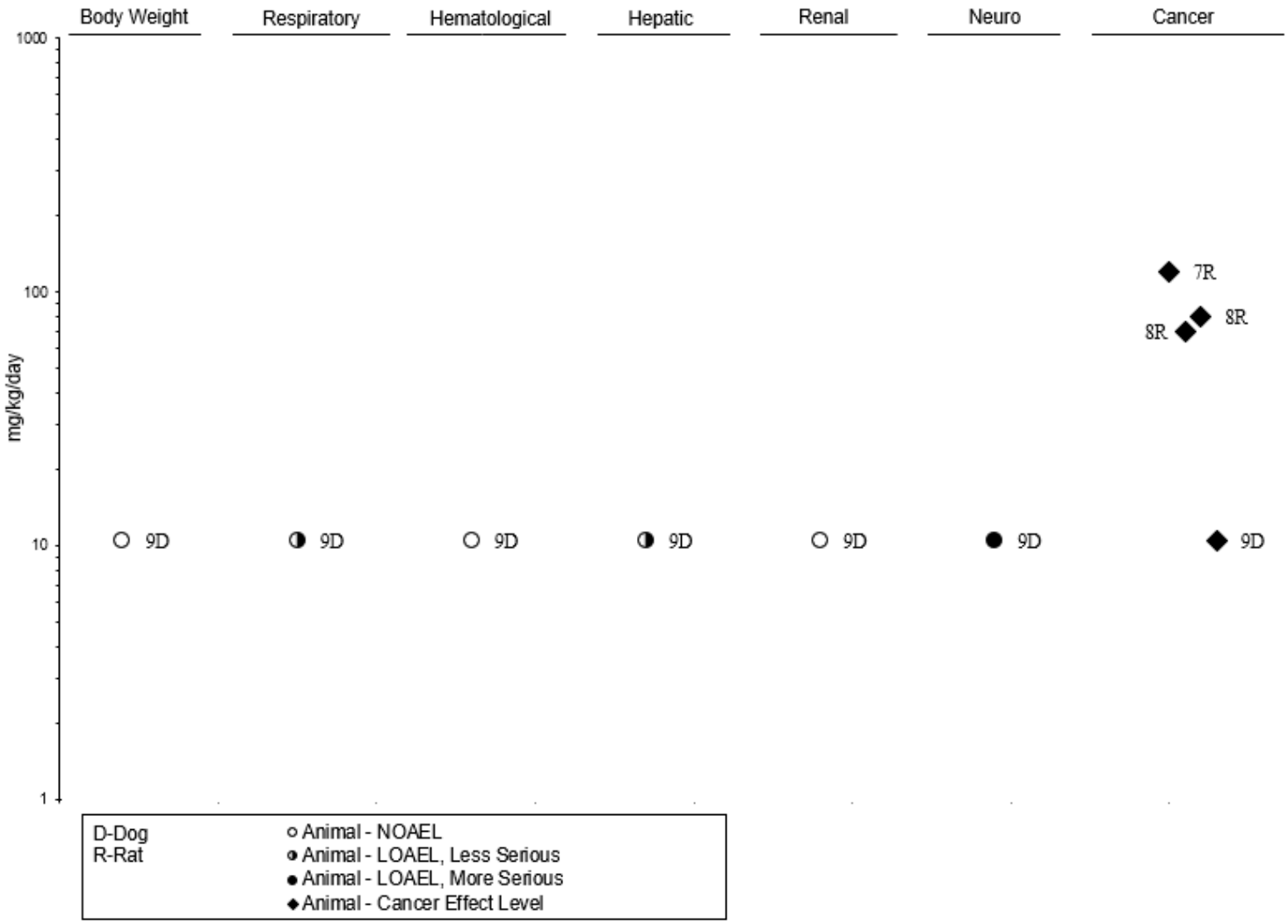


Figure 2-2. Levels of Significant Exposure to 3,3'-Dichlorobenzidine – Oral

Chronic (≥365 days)



2.2 DEATH

No studies were located describing lethal effects in humans after inhalation, oral, or dermal exposure. No fatalities were observed in rats observed for 14 days following a 1-hour exposure to an unspecified concentration of 3,3'-dichlorobenzidine dihydrochloride dust (Gerarde and Gerarde 1974). No deaths were reported in male rats exposed to 23,700 mg/m^3 3,3'-dichlorobenzidine base (dust) for 2 hours/day for 7 days (Gerarde and Gerarde 1974).

In rats, the acute-duration oral LD_{50} (lethal dose, 50% killed) for 3,3'-dichlorobenzidine free base administered in pure olive oil was estimated to be 7,070 mg/kg, whereas the LD_{50} for a 20% suspension of the dihydrochloride salt in corn oil was 3,820 mg/kg (Gerarde and Gerarde 1974). The cause of death was not discussed. Given this high LD_{50}, acute lethality in humans following oral exposure is unlikely. The minimum dermal lethal dose for 3,3'-dichlorobenzidine (free base) for male and female New Zealand albino rabbits with skin intact was reported to be >8,000 mg/kg (Gerarde and Gerarde 1974). The cause of death was not discussed.

A single dose oral lethality study reported LD_{50} values in male and female mice of 488 and 676 mg/kg, respectively. The reported LD_{50} values for a daily dose given over 7 days to male and female mice were 352 and 386 mg/kg/day, respectively (Gaines and Nelson, as cited in EPA 1980a).

2.3 BODY WEIGHT

No human studies have evaluated the effect of 3,3'-dichlorobenzidine exposure on body weight. Animal studies are limited to one study examining the oral route. No significant changes in body weight were seen in female beagle dogs exposed to 10.4 mg/kg/day of 3,3'-dichlorobenzidine for 7 years, compared to controls over the same period (Stula et al. 1978).

2.4 RESPIRATORY

No human studies were located examining respiratory effects following inhalation, oral, or dermal exposure to 3,3'-dichlorobenzidine.

In animals, respiratory effects have not been directly attributed to inhalation or oral exposure to 3,3'-dichlorobenzidine. No adverse health effects were observed in male rats exposed by inhalation to

3,3'-dichlorobenzidine free base (23,700 mg/m^3) 2 hours/day for 7 days (Gerarde and Gerarde 1974). In another study, 10 rats were exposed to an unspecified concentration of 3,3'-dichlorobenzidine dihydrochloride dust particles for 1 hour and then observed for 14 days. Slight-to-moderate pulmonary congestion and one pulmonary abscess were observed upon necropsy (Gerarde and Gerarde 1974). The effects observed in the study using the ionized (hydrochloride) form of 3,3'-dichlorobenzidine may have been due to the irritative properties of hydrochloric acid released from the salt in combination with particulate toxicity. Dyspnea was observed in one of six female dogs exposed to 10.4 mg/kg/day 3,3'-dichlorobenzidine for 6.6 years, which likely resulted as a secondary effect of liver disease that this dog was experiencing. No respiratory effects were observed in any other dogs, including controls (Stula et al. 1978). No studies were located regarding respiratory effects in animals after dermal exposure to 3,3'-dichlorobenzidine.

2.5 CARDIOVASCULAR

No studies were located regarding cardiovascular effects in humans and animals after exposure to 3,3'-dichlorobenzidine.

2.6 GASTROINTESTINAL

No studies were located regarding gastrointestinal effects in humans or animals after exposure to 3,3'-dichlorobenzidine.

2.7 HEMATOLOGICAL

No studies were located regarding hematological effects in humans after exposure to 3,3'-dichlorobenzidine.

Evidence in animals is limited to one oral exposure study in dogs. Hematological parameters (erythrocyte count, hemoglobin concentration, hematocrit, and leukocyte count) were found to be normal in dogs exposed to 10.4 mg/kg/day 3,3'-dichlorobenzidine for 7 years (Stula et al. 1978).

2.8 MUSCULOSKELETAL

No studies were located regarding musculoskeletal effects in humans or animals after exposure to 3,3'-dichlorobenzidine.

2.9 HEPATIC

No human studies have evaluated the hepatic toxicity of 3,3'-dichlorobenzidine from any exposure route.

Limited animal evidence suggests that chronic-duration oral exposure to 3,3'-dichlorobenzidine results in mild-to-moderate liver injury. Six female beagle dogs were administered 3,3'-dichlorobenzidine (100 mg in gelatin capsules; 10.4 mg/kg body weight mean dose) 3 times/week for 6 weeks, and then 5 times/week for up to 7.1 years (Stula et al. 1978). All had modestly elevated plasma alanine aminotransferase (ALT) activity during the first 3 years of a 7-year treatment period. Thereafter, ALT levels returned to normal in three of the experimental animals, and two remained elevated for the duration of the study. Elevated ALT levels may have been due to the exposure to 3,3'-dichlorobenzidine that resulted in chronic hepatic injury in these dogs that ultimately led to development of liver tumors. One of the six dogs, sacrificed after 42 months of the test, showed a marked fatty change in the liver. None of the six control dogs exhibited adverse liver effects.

2.10 RENAL

No human studies have evaluated the renal toxicity of 3,3'-dichlorobenzidine.

Urinary parameters (urobilinogen, pH, osmolality, volume, protein, sugar, and sediment) were normal in female beagle dogs orally exposed to 3,3'-dichlorobenzidine (10.4 mg/kg/day) daily in a 7-year study. At necropsy, no histological effects to the kidneys were reported in any of the dogs (Stula et al. 1978).

2.11 DERMAL

No studies were located regarding dermal effects in humans or animals after inhalation or oral exposure to 3,3'-dichlorobenzidine.

Dermatitis was cited as the only verified health problem encountered by workers in contact with the free base of 3,3'-dichlorobenzidine in a manufacturing plant of the chemical (Gerarde and Gerarde 1974). There was no discernable skin irritation when 3,3'-dichlorobenzidine dihydrochloride (at an unstipulated dose) was applied to the intact and abraded skin of rabbits (Gerarde and Gerarde 1974). Similarly, an aqueous suspension of 3,3'-dichlorobenzidine instilled intradermally into rats at a dose of 700 mg/kg did not produce adverse effects (Gerarde and Gerarde 1974). The observations in humans may have been allergic dermatitis, and specific protocols are required to make these determinations in laboratory animals.

2.12 OCULAR

No studies were located regarding ocular effects in humans after inhalation, oral, or dermal exposure to 3,3'-dichlorobenzidine.

Studies examining ocular toxicity in animals were limited to the dermal route. No effects were reported in rabbits when 100 mg of 3,3'-dichlorobenzidine (free base) was placed in the conjunctival sac of the eye (Gerarde and Gerarde 1974). The duration of exposure and the vehicle used are not stated. However, 0.1 mL of 3,3'-dichlorobenzidine dihydrochloride in a 20% corn oil suspension produced erythema, pus, and corneal opacity, giving a 76% score in the Draize test within an hour when placed in the conjunctival sac of the eye of the rabbit (Gerarde and Gerarde 1974). This response is likely associated with the release of hydrochloric acid following the salt's contact with the moist surface of the eye.

2.13 ENDOCRINE

No studies were located regarding endocrine effects in humans or animals after oral, inhalation, or dermal routes of exposure to 3,3'-dichlorobenzidine.

An *in vitro* screening assay indicated that 3,3'-dichlorobenzidine had the highest antagonist potency and the capability of binding to the androgen receptor (Araki et al. 2005). The assay evaluated androgen receptor agonist and antagonist activity using an androgen receptor transcriptional activation assay and an *in vitro* androgen receptor binding assay. The study authors reported that 3,3'-dichlorobenzidine inhibited dihydrotestosterone-induced transcriptional activation and was higher than the anti-androgenic potency of *p,p'*-DDE, *o,p'*-DDT, and linuron (Araki et al. 2005).

2.14 IMMUNOLOGICAL

No studies were located regarding immunological effects in humans or animals after oral, inhalation, or dermal exposure to 3,3'-dichlorobenzidine.

2.15 NEUROLOGICAL

No studies were located regarding neurological effects in humans after oral, inhalation, or dermal exposure to 3,3'-dichlorobenzidine.

In a carcinogenicity study, one of six dogs exhibited convulsions after 21, 28, or 42 months of oral treatment with 10.4 mg/kg/day of 3,3'-dichlorobenzidine over a period of 3.5 years (Stula et al. 1978). Necropsy at 42 months revealed slight neuronal degeneration in the convulsing dog; although the specific location was not indicated, histological examination was performed on the brain and spinal cord. No neurological effects were observed in any other dogs, including controls. No further studies examined neurological effects in animals by any exposure route.

No studies were located regarding the following three health effects in humans or animals after inhalation, oral, or dermal exposure to 3,3'-dichlorobenzidine:

2.16 REPRODUCTIVE

2.17 DEVELOPMENTAL

2.18 OTHER NONCANCER

2.19 CANCER

Seven epidemiological studies were identified. One study reported no association between exposure to 3,3'-dichlorobenzidine and increase in incidence or death due to bladder cancer (Gerarde and Gerarde 1974). Two studies identified no cases of bladder cancer among workers in occupational settings where exposure to 3,3'-dichlorobenzidine was exclusive (Gadian 1975; MacIntyre 1975); however, cases of bladder cancer were reported among workers exposed to a mixture of benzidine and 3,3'-dichloro-benzidine (Gadian 1975). These studies had limited power to detect an exposure-related effect due to small sample size, short follow-up time, no control group, or co-exposure to other chemicals. While both Myslak et al. (1991) and Ouellet-Hellstrom and Rench (1996) reported an increase in bladder tumors in

workers exposed to either benzidine-based azo dyes (Myslak et al. 1991) or arylamines (Ouellet-Hellstrom and Rench 1996), a direct attribution to solely 3,3'-dichlorobenzidine cannot be made.

Rosenman and Reilly (2004) identified 22 bladder cancer cases among a cohort of 538 workers exposed to benzidine and/or 3,3'-dichlorobenzidine from a chemical manufacturing plant. The plant produced benzidine from 1960 to 1972 and 3,3'-dichlorobenzidine from 1961 to 2001. The employees who had worked at the facility between 1960 and 1977 were identified. SMRs were significantly increased for all cancers (in workers exposed to both chemicals): 1.54 (95% CI 1.04–2.19); bladder cancer: 8.34 (95% CI 1.72–24.78); and lymphohematopoietic cancer: 2.84 (95% CI 1.04–6.18). Of the 538 workers, 202 had exposure to 3,3'-dichlorobenzidine alone. Only one case of bladder cancer was identified among those workers, while three employees died from lymphohematopoietic cancer (SMR 6.62, 95% CI 1.37–19.36). Limitations of this study include lack of smoking status data and no measure of exposure other than duration of work. A follow-up study examined 488 members of this cohort through 2014 (Millerick-May et al. 2021). Among workers exposed to benzidine and 3,3'-dichlorobenzidine, an association was observed between exposure and bladder cancer on the standard incidence ratio (SIR) of 3.11 (95% CI 1.97–4.67) and SMR of 1.12 (95% CI 1.12–10.50). In contrast, for workers exposed to 3,3'-dichloro-benzidine alone, no associations were observed between exposure and the bladder cancer (SIR 0.89, 95% CI 0.11–3.23; SMR 0.07, 95% CI 0.07–16.15).

In animal studies, bladder tumors have also been observed in rats and dogs treated with 3,3'-dichloro-benzidine (Pliss 1959; Stula et al. 1975). Oral exposure to 3,3'-dichlorobenzidine caused tumors in several animal species at several tissue sites. An increased incidence in hepatomas or hepatic tumors has been reported in mice (Osanai 1976; Pliss 1959). Tumors were observed in rats at a variety of sites, including the Zymbal gland, mammary gland, bladder, hematopoietic system, skin, ileum, sebaceous glands, salivary gland, liver, kidney, thyroid, and papillomas of the bladder (Pliss 1959). Pliss (1959) reported high mortality among exposed rats, and 10/29 rats that developed tumors were responsible for the various locations reported. Stula et al. (1975) administered 3,3'-dichlorobenzidine to 50 male and 50 female ChR-CD rats at a dose of 50 mg/kg/day in the diet. Control groups of 50 males and 50 females were also included in the study. The planned study duration was 2 years; however, the average number of days on the test was 349 days (range of 143–488 days) for females and 353 days (range of 118–486) for males. No reason for early mortality was provided. Statistically significant increases in tumor incidences were reported for males: granulocytic leukemia, mammary adenocarcinoma, and Zymbal gland carcinoma. The only tumors that showed a statistically significant increase in females were mammary adenocarcinomas (Stula et al. 1975).

Stula et al. (1978) reported hepatocellular carcinomas (67% incidence) and papillary transitional cell carcinomas of the bladder (83%) in female dogs fed approximately 10.4 mg/kg/day orally in gelatin capsules over a period of 6.6–7.1 years. Although a small number of dogs (6) were evaluated, and only one sex and one dose were used, the significant increase in tumor rate in this group of dogs demonstrates the carcinogenicity of this chemical in this species.

A synergistic role for 3,3'-dichlorobenzidine in the development of bladder cancer has been suggested. This was proposed in a study in which no carcinomas were found in any rats administered one of the following: 0.03% 3,3'-dichlorobenzidine in the diet, 0.001% BBN (N-butyl-N-(hydroxybutyl) nitrosamine) in drinking water, 0.0005% 2-acetylaminofluorene (2-AAF) in the diet, or 0.04% N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT) in the diet for a period of 40 weeks (Ito et al. 1983). However, when BBN plus 3,3'-dichlorobenzidine were fed together at the same dose levels as above, there was a marked increase in the presence of papillary or nodular hyperplasia in the rat bladder, and the appearance of one papilloma. Based on these findings, the authors suggested that 3,3'-dichloro-benzidine had a synergistic effect on the carcinogenicity of BBN. In rats sequentially administered BBN (0.01%), FANFT (0.15%), 2-AAF (0.025%), and 3,3'-dichlorobenzidine (0.03%) for 4 weeks each, the incidence of bladder cancer after administration of the four chemicals was no different than after administration of the first three, suggesting no interactive effect of any type for 3,3'-dichlorobenzidine (Ito et al. 1983).

Saffiotti et al. (1967) did not find carcinogenic effects or changes in bladder pathology in Syrian hamsters fed a lifetime diet of 3,3'-dichlorobenzidine. No bladder carcinomas were observed in rats exposed to 27 mg/kg/day for 4 or 40 weeks (Ito et al. 1983), nor were any mammary tumors observed in rats administered approximately 49 mg/kg/day of 3,3'-dichlorobenzidine dihydrochloride by gavage once every 3 days over a 30-day period and sacrificed 8 months later (Griswold et al. 1968).

The Cancer Effect Level (CEL) (i.e., lowest dose that produced a tumorigenic response for each species) and the duration category of exposure to 3,3'-dichlorobenzidine are shown in Table 2-2 and plotted in Figure 2-2. Based on the increased incidence in mammary adenocarcinomas in rats reported in the Stula et al. (1975) study, EPA (IRIS 2006) calculated an oral slope factor of 0.45 per (mg/kg)/day. Doses corresponding to risk levels ranging from 10^{-4} to 10^{-7} are $2.2x10^{-4}$ to $2.2x10^{-7}$ mg/kg/day, respectively.

No studies were located regarding cancer effects in animals after inhalation or dermal exposure to 3,3'-dichlorobenzidine.

The U.S. Department of Health and Human Services classifies 3,3'-dichlorobenzidine as reasonably anticipated to be a human carcinogen based on sufficient evidence in animals; inadequate data from epidemiological studies (NTP 2016). EPA classifies it as B2; probable human carcinogen based on evidence in rats, mice, and dogs; inadequate human data (IRIS 2006). The International Agency for Research on Cancer (IARC) classifies 3,3'-dichlorobenzidine as possibly carcinogenic to humans (Group 2B) based on sufficient evidence in animals; inadequate evidence in humans (IARC 1987).

2.20 GENOTOXICITY

As summarized in Table 2-3, 3,3'-dichlorobenzidine appeared genotoxic in most *in vitro* test systems employed. *In vitro* tests using *Salmonella typhimurium* have produced mixed results. One study (Lazear et al. 1979) reported negative results for gene mutation both with and without activation in the TA100 strain of *S. typhimurium* and positive results in the TA98 strain with and without activation. Five studies reported positive results for gene mutation both with and without activation in two strains of *S. typhimurium* (Chung et al. 2000; Lazear et al. 1979; Makena and Chung 2007; Savard and Josephy 1986; Wang et al. 2005). Four studies reported positive gene mutation results only when activation was applied (Chung et al. 2000; Claxton et al. 2001; Vithayathil et al. 1983; Wang et al. 2005). The results presented by Claxton et al. (2001) were weakly mutagenic and light was used as the activation method in Wang et al. (2005). Imaoka et al. (1997) reported DNA damage in *S. typhimurium* NM2009 after incubation with 3,3'-dichlorobenzidine activated by mouse kidney or bladder microsomes or rat liver microsomes. DNA damage was reported in human cell lines; one cell line that did require activation and three that did not require activation (Chen et al. 2003, 2014; Hering et al. 2018; Wang et al. 2005). Shiraishi (1986) reported an increase in sister chromatid exchange frequency in two cell lines. One of the cell lines was positive with and without activation; the other was positive only with activation (Shiraishi 1986). 3,3'-Dichlorobenzidine formed adducts with calf thymus DNA when incubated with rat liver S9 (Bratcher and Sikka 1982).

Table 2-3. Genotoxicity of 3,3'-Dichlorobenzidine *In Vitro*

Species (test system)	Endpoint	Activation system	Results Activation With	Activation Without	Reference
Prokaryotic organisms					
Salmonella typhimurium TA98	Gene mutation	Mouse liver S9	+	+	Lazear et al. 1979
S. typhimurium TA98	Gene mutation	Hamster liver S9	+	+	Savard and Josephy 1986
S. typhimurium TA98	Gene mutation	Rat liver S9	+	ND	Vithayathil et al. 1983
S. typhimurium TA100	Gene mutation	Mouse liver S9	–	–	Lazear et al. 1979
S. typhimurium NM2009	DNA damage	Mouse kidney S9	+	ND	Imaoka et al. 1997
S. typhimurium NM2009	DNA damage	Mouse bladder S9	+	ND	Imaoka et al. 1997
S. typhimurium NM2009	DNA damage	Mouse kidney CYP4B1	+	ND	Imaoka et al. 1997
S. typhimurium NM2009	DNA damage	Rat liver CYP4B1	+	ND	Imaoka et al. 1997
S. typhimurium TA7004	Gene mutation	Hamster liver S9	(+)	ND	Claxton et al. 2001
S. typhimurium TA102	Gene mutation	Rat liver S9	+	+	Makena and Chung 2007
S. typhimurium TA98	Gene mutation	Rat liver S9	+	+	Chung et al. 2000
S. typhimurium TA100	Gene mutation	Rat liver S9	+	–	Chung et al. 2000
S. typhimurium TA102	Gene mutation	Rat liver S9	+	+	Wang et al. 2005
S. typhimurium TA102	Gene mutation	Light	+	–	Wang et al. 2005
Mammalian cells					
B-lymphoblastoid cell line II	Sister chromatid exchange	Rat liver S9	+	–	Shiraishi 1986
B-lymphoblastoid cell line III	Sister chromatid exchange	Rat liver S9	+	+	Shiraishi 1986
Human lymphocytes (comet assay)	DNA damage	N/A	ND	+	Chen et al. 2003
Human Jurkat T cells (comet assay)	DNA damage	Light	+	–	Wang et al. 2005
Human HaCaT (keratinocyte cancer cells) and BJ (fibroblast) skin cell lines	DNA damage	NA	ND	+	Hering et al. 2018
HepG2 cells (Comet Assay)	DNA damage	NA	ND	+	Chen et al. 2014
Calf thymus	DNA binding	Rat liver S9	ND	+	Bratcher and Sikka 1982

– = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid; NA = not applicable; ND = not determined

In vivo genotoxicity has been evaluated in rats and mice (Table 2-4). Micronuclei were induced in polychromatic erythrocytes of the liver of fetal mice exposed transplacentally to 3,3'-dichlorobenzidine,

and in liver cells of adult male mice treated orally with 3,3'-dichlorobenzidine at a maximum tolerated dose reported to be 1,000 mg/kg (Cihak and Vontorkova 1987). A sex difference in the genotoxicity of the compound is suggested, since adult male mice, but not pregnant females developed erythrocyte micronuclei following 3,3'-dichlorobenzidine exposure. 3,3'-Dichlorobenzidine given in single intraperitoneal doses of 125, 250, and 500 mg/kg or double intraperitoneal doses of 0, 75, 150, and 300 mg/kg did not result in micronucleated reticulocyte induction in the peripheral blood of CD-1 male mice (Morita et al. 1997). Gavage of 3,3'-dichlorobenzidine at doses of 67.5, 125, and 250 mg/kg did not result in micronucleated reticulocyte induction in the peripheral blood of CD-1 male mice (Morita et al. 1997). However, MS/Ae mice (male and female) did show micronucleated reticulocyte induction after double intraperitoneal injection (doses of 120, 180, and 270 mg/kg), but these responses were weak. Bone marrow examination was not reported in this study.

Table 2-4. Genotoxicity of 3,3'-Dichlorobenzidine *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Mouse bone marrow (male)	Micronuclei	+	Cihak and Vontorkova 1987
Mouse bone marrow (female)	Micronuclei	–	Cihak and Vontorkova 1987
Mouse fetal liver	Micronuclei	+	Cihak and Vontorkova 1987
Rat liver cells (male)	Unscheduled DNA synthesis	+	Ashby and Mohammed 1988
Mouse (male)	DNA binding	+	Ghosal and Iba 1990
Rat (male)	DNA binding	+	Ghosal and Iba 1990
CD-1 mouse peripheral blood (male)	Micronuclei	–	Morita et al. 1997
MS/Ae mice (male and female) peripheral blood	Micronuclei	(+)	Morita et al. 1997
Male ddY mice eight organs (stomach, colon, liver, kidney, bladder, lung, brain, bone marrow)	DNA damage	+ in six of eight organs tested	Sasaki et al. 1999

– = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid

Ashby and Mohammed (1988) reported a positive finding for unscheduled DNA synthesis in rats. Single oral administration of 20 or 100 mg/kg radiolabeled 3,3'-dichlorobenzidine to male Sprague-Dawley rats or Swiss-Webster mice resulted in extensive binding of the compound to tissue (liver, bladder, and intestine) DNA 12, 24, or 96 hours, and 9 or 14 days after treatment (Ghosal and Iba 1990).

Sasaki et al. (1999) administered a single gavage to four male mice at the maximum tolerated dose, which was set at about half the LD_{50} (300 mg/kg), and animals were sacrificed at 0 (zero-time control group), 3,

8, and 24 hours after treatment. Differences in length of DNA migration between control (zero-time) groups vs. other groups were measured with the comet assay and tested for statistical significance. Among cells of eight organs examined (stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow), 3,3'-dichlorobenzidine induced a statistically significant increase in DNA damage in the stomach, liver, bladder, lung, brain, and bone marrow compared with controls. Despite the mixed results, the data provides evidence that 3,3'-dichlorobenzidine is genotoxic. However, data are insufficient to establish a threshold for genotoxicity of 3,3’-dichlorobenzidine.

CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Limited studies exist on the toxicokinetics of 3,3'-dichlorobenzidine in humans. Most of the available information is on urinary elimination of the compound following occupational exposure. These data are summarized below.

- Evidence from animal studies suggests that 3,3'-dichlorobenzidine is rapidly absorbed from the gastrointestinal tract.
- Animals administered a single oral dose of [^{14}C]-3,3'-dichlorobenzidine showed highest concentrations of radioactivity in the liver, kidney, lung, spleen, heart, pancreas, and testes.
- In rats, a major step in the elimination of 3,3'-dichlorobenzidine is metabolic transformation. N-Acetyl metabolites (N-acetyl-3,3'-dichlorobenzidine and N,N'-diacetyl-3,3'-dichlorobenzidine) have been detected in urine of rats. N-acetyl metabolites are formed *in vivo* by hepatic N-acetyltransferase(s).
- Studies in laboratory animals show that the primary excretory route for orally administered 3,3'-dichlorobenzidine is the bile and feces.
- In humans, some isozyme(s) of N-acetyltransferase show marked polymorphic differences; it is thus possible that the proportion of the dose of 3,3'-dichlorobenzidine converted to its N-acetyl metabolites in humans may vary widely between individuals. The metabolites undergo rapid excretion primarily in urine and to a lesser extent in feces. Unchanged 3,3'-dichlorobenzidine occurs as a minor urinary excretion product.

3.1.1 Absorption

Human absorption data for 3,3'-dichlorobenzidine are limited. 3,3'-Dichlorobenzidine has been detected in the urine of workers in facilities using 3,3'-dichlorobenzidine under conditions that favored inhalation of 3,3'-dichlorobenzidine-bound particulate matter (Meigs et al. 1954; NIOSH 1986a, 1986b). Under these conditions, it is reasonable to expect that some of the 3,3'-dichlorobenzidine found in the urine could have resulted from pulmonary absorption. However, conditions in the plants were also conducive to dermal absorption. Therefore, some of the 3,3'-dichlorobenzidine dose found in the urine could have come from dermal exposure. In addition, since the mucociliary clearance mechanism moves larger particulates (5–10 μm) out of the lungs into the gastrointestinal tract, it is reasonable to expect that some gastrointestinal dose was received as well.

A study in volunteers found acetylated metabolites in the urine 24 hours after a single 250 mg oral dose of 3,3'-dichlorobenzidine, which indicated that the compound is absorbed (Belman et al. 1968). No studies were located regarding absorption of 3,3'-dichlorobenzidine following dermal exposure in humans. Because of large particle size and increased usage of closed systems and protective clothing, dermal absorption would be minimized.

No information was located on absorption in animals following inhalation exposure. In animals, absorption of 3,3'-dichlorobenzidine from the gastrointestinal tract is rapid. Following a dose of 40 mg/kg, the plasma level of unchanged 3,3'-dichlorobenzidine attained a peak concentration of 1.25 µg/mL at 4 hours in Sprague-Dawley rats. Further, about 90% of the administered radioactivity was excreted in feces (via bile) and urine within 72 hours largely as metabolites. The elimination is biphasic, with half-lives of 6 and 14 hours in plasma for the rapid and slow phases, respectively (Hsu and Sikka 1982).

In animals, dermally applied 3,3'-dichlorobenzidine (in acetone) is moderately absorbed. Based on the amount of radioactivity remaining at the site of application, the extent of dermal absorption of applied [^{14}C]-3,3'-dichlorobenzidine to the shaved skin of rats at 1, 8, and 24 hours following the application was estimated to be 6, 23, and 49%, respectively (Shah and Guthrie 1983).

3.1.2 Distribution

No studies were located regarding distribution of 3,3'-dichlorobenzidine in humans following inhalation, oral, or dermal exposure. There were no studies in animals regarding distribution following inhalation.

In animals, following oral exposure, 3,3'-dichlorobenzidine appears to be widely distributed. The distribution of radioactivity in male rat tissues after the oral administration of [^{14}C]-3,3'-dichlorobenzidine has been studied (Hsu and Sikka 1982). Maximum plasma radioactivity was found 8 hours after administration. Twenty-four hours after a single oral dose the radioactivity was widely distributed with the highest levels of radioactivity found in the liver, followed by the kidney, lung, spleen, heart, pancreas, and testes. After 96 hours, tissues that retained ≥0.02% of the administered radioactivity were: liver (1.48%), muscle (0.37%), kidney (0.19%), and lung (0.02%). Repeated oral administration of [^{14}C]- 3,3'-dichlorobenzidine (animals received six daily doses) resulted in tissue radioactive levels 3–4 times as high as the radioactivity in tissues of animals that received a single dose. Similarly, the rate of decline of radioactivity in tissues was generally higher in animals that received a single dose than in those

treated with multiple doses. The authors concluded that repeated dosing with 3,3'-dichlorobenzidine did not result in a substantial retention of ^{14}C, and the compound may be considered to have a fairly low tendency to accumulate in tissues following repetitive dosing (Hsu and Sikka 1982).

The distribution of [^{14}C]-3,3'-dichlorobenzidine in adult male Fisher rat tissues following dermal administration was studied by Shah and Guthrie (1983). Tissues retaining >0.1% of the administered radioactivity 24 hours after application were liver (4.09%), blood (0.75%), and lung (0.45%). The level in the lung was the same at the 8- and 24-hour time points. Differences in the tissue distribution pattern of total radioactivity between the oral and dermal routes of 3,3'-dichlorobenzidine administration may be presumed to reflect differences in the rates of absorption from these sites. Additionally, tissue distribution patterns depend on blood flow, as ingested 3,3'-dichlorobenzidine is absorbed from the gastrointestinal tract and enters portal venous flow; however, this would not be expected following dermal exposure. These differences suggest that the target organ in which 3,3'-dichlorobenzidine exerts an adverse effect may depend on the route of exposure to the compound. Organ toxicity can be better evaluated in comparative studies designed to test tissue distribution and persistent exposure.

There is indirect evidence that 3,3'-dichlorobenzidine or its metabolites can cross the placenta. A study that examined the potential genotoxic effects of 3,3'-dichlorobenzidine found that oral administration of 3,3'-dichlorobenzidine to pregnant rats induced micronuclei in the liver of fetuses (Cihak and Vontorkova 1987). There is no information regarding accumulation of 3,3'-dichlorobenzidine or metabolites in breast milk or its potential transfer to offspring via breast milk.

3.1.3 Metabolism

No studies were located regarding metabolism in humans after inhalation or dermal exposure to 3,3'-dichlorobenzidine. One study by Lee et al. (2003) examined DNA adducts of workers from a dye manufacturing plant. Limited details on the workplace exposure pathways are given in the study. No studies were located regarding metabolism in animals after inhalation exposure to 3,3'-dichlorobenzidine.

Information from a study in which four volunteers ingested a single 250 mg dose of 3,3'-dichloro-benzidine suggests that this chemical undergoes N-acetylation, and that metabolites may be excreted in the urine either free or as glucuronides (Belman et al. 1968). N-Acetylation appears to be the major path for the metabolism of 3,3’-dichlorobenzidine in mammals (Iba 1987, 1989; Lazear et al. 1979; Reid et al. 1984; Tanaka 1981).

There is no information regarding the metabolism of 3,3'-dichlorobenzidine in children. However, N-acetylation in humans is likely mediated by one of two families of N-acetyltransferases. One of these families, NAT2, is developmentally regulated (Suchy 2014). Some enzyme activity can be detected in the fetus by 16 weeks of gestation, and all infants exhibit the slow acetylator phenotype between birth and 55 days of age. By 3 years of age, NAT2 appears fully determined as phenotype expression distribution appears similar to that of adult populations (Suchy 2014). Also, uridine 5'-diphospho-glucuronosyltransferase (UGT), responsible for the formation of glucuronide conjugates, seems to achieve adult capacity by 2–6 months of age, but may not fully mature until up to 30 months in some individuals (Suchy 2014). These data suggest that metabolism of 3,3'-dichlorobenzidine by infants will differ from that in adults in extent, rate, or both.

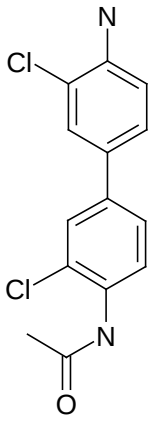
Studies in animals also indicate that 3,3'-dichlorobenzidine is extensively metabolized. Bile and urine of rats given single oral doses of [^{14}C]-3,3'-dichlorobenzidine (40 mg/kg/day) contained five metabolites of 3,3'-dichlorobenzidine in addition to the parent compound. However, none of the metabolites were identified, but most were reported to be conjugates (Hsu and Sikka 1982).

A 24-hour urine sample of rats given a single oral dose of 3,3'-dichlorobenzidine (50 mg/kg/day) contained unchanged 3,3'-dichlorobenzidine, N,N'-diacetyl-3,3'-dichlorobenzidine, and N-acetyl-3,3'-dichlorobenzidine in a ratio of 1:3:10 (Tanaka 1981). Chemical structures of these acetylated 3,3'-dichlorobenzidine metabolites are presented in Figure 3-1. Indirect evidence for the formation of nitroso derivatives was found in a study in which 3,3'-dichlorobenzidine was administered to female Wistar rats by gavage (Birner et al. 1990). This was due to the fact that an amine could be extracted after hydrolysis of the hemoglobin adducts. The authors stated that the most likely process by which the adducts were formed was a reaction between a nitroso derivative of 3,3'-dichlorobenzidine and sulfhydryl in cysteine residues of hemoglobin.

These metabolites could arise either by direct N-oxidation of the amino group or by deacetylation of the hydroxamic acid. A potential form of the nitroso intermediate is shown in Figure 3-2. It is hypothesized the nitroso-intermediate of 3,3'-dichlorobenzidine could be formed from oxidation of the hydroxyl-intermediates N-hydroxy-N'-acetyl-dichlorobenzidine and N-hydroxy-dichlorobenzidine, which are both speculated to be biologically active (Birner et al. 1990). These hydroxyl derivatives are shown in Figure 3-3.

Figure 3-1. Chemical Structures of 3,3'-Dichlorobenzidine Metabolites

N-acetyl-3,3'-dichlorobenzidine



N,N'-diacetyl-3,3'-dichlorobenzidine

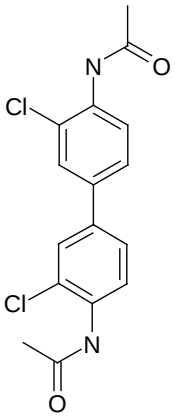


Figure 3-2. Potential Form of the Nitroso Intermediate

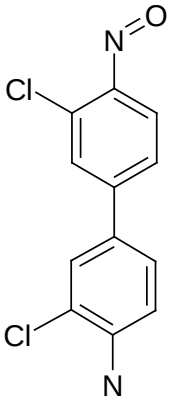
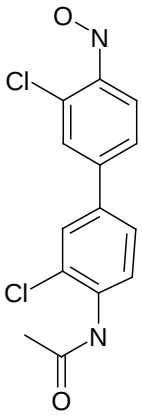
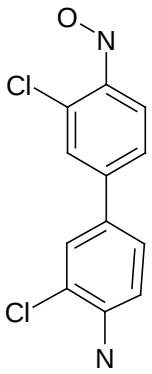


Figure 3-3. Hydroxy- Metabolites of 3,3'-Dichlorobenzidine

N-Hydroxy-N'-acetyl-dichlorobenzidine



N-Hydroxy-dichlorobenzidine



In a 24-hour urine sample of rats given a single dermal application of 3,3'-dichlorobenzidine (50 mg/kg/day), N,N'-diacetyl-3,3'-dichlorobenzidine (but not N-acetyl-3,3'-dichlorobenzidine or the unchanged chemical) was detected (Tanaka 1981). Since the mutagenicity of diacetylated product is much less than either the monoacetylated or parent compound (Lazear et al. 1979; Reid et al. 1984; Tanaka 1981), diacetylation appears to be a detoxification reaction for 3,3'dichlorobenzidine.

The metabolism of several 3,3'-dichlorobenzidine-based pigments has been studied in animal experiments to determine if they are metabolized to 3,3'-dichlorobenzidine. In a study where rats were exposed by inhalation to Pigment Yellow 17 (230 mg/m^3 air) for 4 hours, 3,3'-dichlorobenzidine was not detected in either urine or blood during the following 14 days (Hofmann and Schmidt 1993). No detectable residues of 3,3'-dichlorobenzidine were found in urine samples of hamsters administered a single dose of 100 mg/kg purified Yellow 12 (NCTR 1979; Nony et al. 1980). Similarly, 3,3'-dichlorobenzidine was not detected in urine samples of rats fed 3,3'-dichlorobenzidine-derived pigments (C.I. Pigment Yellow 12, 16, and 83) in the diet at concentrations of 0.1% (1,000 ppm), 0.3% (3,000 ppm), and 0.9% (9,000 ppm) for 104 weeks (Leuschner 1978). Based on the results of these studies, there is no evidence for the metabolic cleavage of tested pigments to 3,3'-dichlorobenzidine in test animals (Hofman and Schmidt 1993; Leuschner 1978; NCTR 1979; Nony et al. 1980).

The N-oxidation of 3,3'-dichlorobenzidine may lead to DNA adducts and subsequently to DNA lesions and mutations. It is not clear from the literature exactly which metabolites of 3,3'-dichlorobenzidine react to form adducts with hemoglobin and DNA. Zwirner-Baier and Neumann (1998) analyzed hydrolysable hemoglobin adducts following oral administration of 3,3'-dichlorobenzidine to female Wistar rats. The results showed that deamination did not take place (low adduct levels were found); the monoacetamide (N-acetyl-3,3'-dichlorobenzidine) was readily deacetylated *in vivo*, whereas the diacetamide (N,N'-diacetyl-3,3'-dichlorobenzidine) was not. In addition, acetylation polymorphism was studied with 3,3'-dichlorobenzidine in slow-acetylating A/J mice and rapid-acetylating C57BL/6J mice (Zwirner-Baier and Neumann 1998). The slow acetylator genotype was associated with significantly higher hemoglobin-adduct levels. The results provide additional support for the role of the acylation pathway in the epidemiological finding of susceptibility of slow acetylators to developing occupational bladder cancer. Zwirner-Baier and Neumann (1998) reported that in Wistar rats the equilibrium between 3,3'-dichloro-benzidine and its metabolite, N-acetyl-3,3'-dichlorobenzidine, was 5:1. Lee et al. (2003) identified the acylated metabolites of 3,3'-dichlorobenzidine as the N,N'-diacetyl-3,3'-dichlorobenzidine and N-acetyl-3,3'-dichlorobenzidine. In a separate study, Lee (2003) measured metabolites of 3,3'-dichlorobenzidine as hydrolyzed DNA adducts from exfoliated urothelial cells collected from the urine of dye workers exposed

to 3,3'-dichlorobenzidine. In this study, N-acetyl-3,3'-dichlorobenzidine and 3,3'-dichlorobenzidine were extracted as the adducts once they were hydrolyzed from the DNA. The detection of the diacylated metabolite (N,N'-diacetyl-3,3'-dichlorobenzidine) was not reported in the study.

Iba (1987) reported mutagenic activity of 3,3'-dichlorobenzidine and concluded that the activation is catalyzed by the cytochrome P450 system. The specific metabolites formed were not characterized; however, Iba (1987) stated that acylation is not required for the activation of 3,3'-dichlorobenzidine, leaving oxidation as an additional activation pathway.

In the case of benzidine, a structurally similar compound, Zwirner-Baier and Neumann (1998) stated, "Martin et al. (1982) identified the DNA-adduct as N-(deoxyguanosin-8-yl)-N'-acetyl-benzidine and proposed the N-hydroxy derivative of the N'-monoacetamide as the proximate genotoxin" (Zwirner-Baier and Neumann 1998, p. 499). It is not definitively known where on DNA 3,3'-dichlorobenzidine derivatives bind to form adducts. Iba (1989) explored the question of where on DNA 3,3'-dichloro-benzidine metabolites bind and found that 3,3'-dichlorobenzidine is metabolized by rat liver microsomes to derivatives that bind covalently to added deoxyguanosine at a neutral pH, but also pointed out that this finding is not consistent with other carcinogenic arylamines. This indicates that the mechanism of binding may be different than with benzidine.

Joppich-Kuhn et al. (1997) found that the hemoglobin adducts formed with 3,3'-dichlorobenzidine are stable *in vivo*, and they persist for the life of the erythrocyte. In the case of DNA adducts, the lifespan of the DNA adducts varied by species and tissue type. Experiments in the liver, bladder epithelium, and small intestinal epithelium of rats and mice following a single oral dose of 3,3'-dichlorobenzidine found that even one dose led to extensive covalent DNA binding, and the rate of adduct removal did not vary between identified target and non-target tissues (Ghosal and Iba 1990). The study authors speculated that peak binding may result in higher rates of carcinogenicity; however, more research is needed to determine this. Specifically, Ghosal and Iba (1990) found that the half-life of the DNA adducts in the liver and in the bladder epithelium were similar in rats and mice (13–14 day) and about 3 times longer than the half-life in the intestinal epithelium.

Studies have further explored the enzymes involved in the metabolism of 3,3'-dichlorobenzidine. Evidence in rats and mice suggests 3,3'-dichlorobenzidine induces hepatic microsomal cytochrome P450 (Iba 1989). Cytochrome P450 inhibitors, α-naphthoflavone and SKF-525A, modified the hepatic microsomal metabolism of 3,3'-dichlorobenzidine. 3,3'-Dichlorobenzidine formed a complex with

oxyferro-P-450 in microsomes, indicating that metabolism occurred. N-Acetyl-3,3'-dichlorobenzidine (acDCB) and azodichlorobenzidine (AzoDCB) were the primary formed metabolites, and are suggested useful indices of 3,3'-dichlorobenzidine metabolism by the study author (Iba 1989). Nicotinamide adenine dinucleotide phosphate (NADPH) enhances 3,3'-dichlorobenzidine covalent binding to polynucleosides in the presence of rat liver S9 *in vitro.* Cytochrome P450 appears involved in the formation of reactive DCB species, as α-naphthoflavone inhibits this NADPH-dependent binding by 50% (Iba 1989).

Iba (1989) suggested that the role of flavin-containing monooxygenase (FMO) in the microsomal metabolism of 3,3'-dichlorobenzidine as N-acetyl-3,3'-dichlorobenzidine is an extractable product with solvents. The role of FMO was examined as carbon monoxide, which does not inhibit FMO activity, and did not appear to affect microsomal metabolism of 3,3'-dichlorobenzidine. In the same study, the study author found that peroxidases do not appear to play a role in metabolism of 3,3'-dichlorobenzidine, despite its roles in the metabolism of most carcinogenic arylamines.

Iba (1989) identified cytochrome P450d, induced by 3,3'-dichlorobenzidine, and FMO as the primary enzymes responsible for forming mutagenic DCB derivates in microsomes. Rat liver microsomes were used as the source of activating enzymes and mutagenicity to TA98. Additional testing showed that cytochrome P450d formed both mutagenic and lipid-binding DCB derivatives. The same testing revealed FMO forms mutagenic, but not lipid-binding, 3,3'-dichlorobenzidine derivates. As similarly described in microsomal metabolism, carbon monoxide did not inhibit microsomal activation of 3,3'-dichloro-benzidine. Additionally, post-oxidative activation does not appear to be important to the hepatic activation of 3,3'-dichlorobenzidine attributed to its mutagenicity in TA98, which requires post-oxidative enzymes, and its activation by rat liver S9 preparation, which does not require these enzymes.

3.1.4 Excretion

Less than 0.2 ppb 3,3'-dichlorobenzidine was detected in urine samples of 36 workers exposed to 3,3'-dichlorobenzidine-derived pigments (Hatfield et al. 1982). However, the study authors did not clearly identify specific pigments. While the study authors did not report the exposure route, workers were likely exposed via inhalation, and dermal exposure may have also occurred.

Very limited information was located regarding excretion of 3,3'-dichlorobenzidine and/or metabolites in humans after oral exposure. In a study of four volunteers who ingested a single 250 mg dose of

3,3'-dichlorobenzidine, the percentage of N-hydroxyacetyl compound excreted free in the urine in 24 hours ranged from 0.32 to 1.55%, whereas the percentage of N-hydroxyacetyl compound excreted as glucuronide in 24 hours ranged from 0.11 to 0.45% (Belman et al. 1968). Studies on the fate of 3,3'-dichlorobenzidine derived pigments fail to provide conclusive evidence that these pigments are broken down to release free 3,3'-dichlorobenzidine in humans.

Results from animal studies show that 3,3'-dichlorobenzidine administered by gavage is excreted primarily in feces and, to a lesser extent, in urine. In rats administered a single oral dose of [^{14}C]-3,3'-dichlorobenzidine (40 mg/kg), the elimination from plasma appeared to be biphasic, with half-lives of about 6 and 14 hours for the rapid and slow phases, respectively (Hsu and Sikka 1982). Elimination of 3,3'-dichlorobenzidine-derived radioactivity from liver, kidneys, and lungs also exhibited rapid and slow phases, with half-lives of 5.8 and 77 hours for the liver, 7.1 and 139 hours for the kidneys, and 3.8 and 43.3 hours for the lungs. These longer half-lives are due to the covalent binding of radiolabeled 3,3'-dichlorobenzidine to tissues. Approximately 58–72% of the administered dose was recovered in bile and feces and 23–33% in urine (Hsu and Sikka 1982). Most of the material found in bile and feces consisted of conjugated metabolites, while most of the material in urine consisted of unconjugated metabolites. No detectable residues of 3,3'-dichlorobenzidine were found in urine samples of hamsters administered a single dose of 100 mg/kg purified Yellow 12 (NCTR 1979; Nony et al. 1980). Similarly, 3,3'-dichlorobenzidine was not detected in urine samples of rats fed 3,3'-dichlorobenzidine-derived pigments (C.I. Pigment Yellow 12, 16, and 83) in the diet at concentrations of 0.1% (1,000 ppm), 0.3% (3,000 ppm), and 0.9% (9,000 ppm) for 104 weeks (Leuschner 1978).

Fecal excretion in rats at 24 hours following 3,3'-dichlorobenzidine dermal exposure was 19% of the administered dose, while urinary excretion accounted for 8% (Shah and Guthrie 1983). Fifty-one percent of the administered dose was unabsorbed from the site of application at 24 hours. The remaining 49% was distributed throughout the body, feces, and urine.

In rats treated orally with 20 mg of 3,3'-dichlorobenzidine/kg body weight over 2 weeks, the urinary excretion rate of the parent compound was nearly constant (Lee et al. 2003). However, the excretion rate of its metabolites, N-acetyl-3,3'-dichlorobenzidine and N,N'-diacetyl-3,3'-dichlorobenzidine, increased during the 14 days they were monitored.

As noted in Section 3.1.3, N-acetylation appears to be a major metabolic path of 3,3'-dichlorobenzidine in mammals. Iba (1989) noted the reduced lipophilicity of 3,3'-dichlorobenzidine effected by N-acetylation,

likely decreasing access to active sites of microsomal oxidases and enhancing bioelimination of 3,3'-dichlorobenzidine (Iba 1989).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan and Andersen 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

No PBPK models were found to have been developed for 3,3'-dichlorobenzidine.

3.1.6 Animal-to-Human Extrapolations

Information on the toxicity of 3,3'-dichlorobenzidine for humans and animals is limited, particularly regarding noncancer endpoints. Therefore, an attempt to discuss potential interspecies differences or similarities in 3,3'-dichlorobenzidine noncancer toxicity based on the limited information available is speculative. 3,3'-Dichlorobenzidine is carcinogenic in animals (Osanai 1976; Pliss 1959, 1963; Stula et al. 1975, 1978). While bladder cancer has been observed in occupational studies, there is no conclusive evidence of carcinogenicity of 3,3'-dichlorobenzidine in humans (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Millerick-May et al. 2021; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996; Rosenman and Reilly 2004); however, there is concern about occupationally exposed subjects because of 3,3'-dichlorobenzidine's structural similarity with the known human and animal carcinogen benzidine. The National Toxicology Program (NTP), EPA, and IARC have concluded that there is sufficient evidence of carcinogenicity in animals, but insufficient evidence in humans.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental

germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to 3,3'-dichlorobenzidine are discussed in Section 5.7, Populations with Potentially High Exposures.

There is some evidence that genetically slow acetylators may be susceptible to bladder cancer from 3,3'-dichlorobenzidine (see Section 3.1.3, Metabolism).

No studies were located that specifically addressed health effects of children from exposure to 3,3'-dichlorobenzidine. No organ or system has been identified as a target for 3,3'-dichlorobenzidine in humans, although dermatitis caused by skin contact with the free base was reported in one study (Gerarde and Gerarde 1974). It is reasonable to assume that the same effect would be seen in children similarly exposed. Because of the structural similarity of 3,3'-dichlorobenzidine with the known human bladder carcinogen benzidine, special attention has been paid to the incidence of bladder cancer among subjects occupationally exposed to 3,3'-dichlorobenzidine. Based on evidence in animal studies, IARC classified 3,3'-dichorobezidine as possibly carcinogenic to humans (Group 2B) (IARC 1987). The U.S. Department of Health and Human Services classifies 3,3'-dichlorobenzidine as reasonably anticipated to be a human carcinogen (NTP 2016). EPA classifies it as B2; probable human carcinogen (IRIS 2006).

No studies were available that provided information on possible adverse developmental effects in humans exposed to 3,3'-dichlorobenzidine. The few available studies in animals were inadequate since they used parenteral administration of high doses of 3,3'-dichlorobenzidine (Golub 1969; Golub et al. 1975; Shabad et al. 1972).

There is no information regarding pharmacokinetics of 3,3'-dichlorobenzidine in children, nor it is known whether 3,3'-dichlorobenzidine can be stored and excreted in breast milk. There have been no direct

measurements in either humans or animals to determine whether 3,3'-dichlorobenzidine can cross the placenta; however, two animal studies provided some indirect evidence that 3,3'-dichlorobenzidine or its metabolites do. In one study, 3,3'-dichlorobenzidine was orally administered to pregnant mice, which resulted in the induction of micronuclei in the liver of fetuses (Cihak and Vontorkova 1987). In another study, pregnant mice were subcutaneously administered 3,3'-dichlorobenzidine, resulting in abnormal growth of the kidneys explanted from the fetuses (Shabad et al. 1972). No information was located on whether 3,3'-dichlorobenzidine can be stored in maternal tissues and be mobilized during pregnancy or lactation, or whether it can reach parental germ cells.

There is no information on the metabolism of 3,3'-dichlorobenzidine in children. Limited data in humans suggest that N-acetylation is an important metabolic pathway (Belman et al. 1968) and a detoxification mechanism. N-Acetylation in humans is likely mediated by one of two families of N-acetyltransferases. One of these families, NAT2, is developmentally regulated (Suchy 2014). Some enzyme activity can be detected in the fetus by 16 weeks of gestation, and all infants exhibit the slow acetylator phenotypes between birth and 55 days of age. By 3 years of age, NAT2 appears fully determined as phenotype expression distribution appears similar to that of adult populations (Suchy 2014). Also, UGT, responsible for the formation of glucuronide conjugates, seems to achieve adult capacity by 2–6 months of age, but may not fully mature until up to 30 months in some individuals (Suchy 2014). These data suggest that metabolism of 3,3'-dichlorobenzidine by infants will differ from that by adults in extent, rate, or both.

No specific references on exposures of infants or children to 3,3'-dichlorobenzidine were located. It is possible that young children may be exposed to 3,3'-dichlorobenzidine by ingesting paint chip debris, painted objects or paints, and soil if the material contains the chemical. Mathematical models (using worst-case assumptions) predicted that the estimated total intake of 3,3'-dichlorobenzidine by infants up to 6 months of age would be $3.6x10^{-8}$ ng/kg body weight/day, about 5 times greater than the estimate of $7.4x\ 10^{-9}$ ng/kg body weight/day for adults ages ≥20 years (Government of Canada 1993).

The adsorption of 3,3'-dichlorobenzidine to soils and sediments is not readily reversible, and the bioavailability of the compound is limited. Therefore, a child who ingested contaminated dirt would be expected to incur less exposure as compared to that from other, more direct routes.

Another potential exposure route for children is through exposure to clothing and tracked-in dirt brought in by parents who work in factories that produce 3,3'-dichlorobenzidine. A public health assessment study conducted in Michigan in 1981 (ATSDR 1996) found the compound in the homes of nine

employees. Samples collected from vacuum cleaner bags had 3,3'-dichlorobenzidine concentrations of up to 10.5 ppm, and dryer lint contained up to 0.074 ppm (ATSDR 1996).

No studies were located that examined possible differential susceptibility between young and older organisms. There are no biomarkers in adults that identify previous childhood exposure. Biomarkers of exposure used for adults can presumably be effective to assess children (see Section 3.3.1).

No information was located regarding either adult or pediatric-specific methods for reducing peak absorption following exposure to 3,3'-dichlorobenzidine, reducing body burden, or interfering with the mechanism of action for toxic effects. In addition, no data were located regarding whether methods for reducing toxic effects of 3,3'-dichlorobenzidine used in adults might be contraindicated in children. There is no information regarding possible transgenerational effects of 3,3'-dichlorobenzidine in humans or animals.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to 3,3'-dichlorobenzidine are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for 3,3'-dichlorobenzidine from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial

cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by 3,3'-dichlorobenzidine are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

3,3’-Dichlorobenzidine and 3,3’-dichlorobenzidine metabolites are in excreted in urine; therefore, urinary levels of 3,3’-dichlorobenzidine and its metabolites are used as biomarkers of exposure. 3,3'-Dichlorobenzidine has been detected in urine of workers (Knoell et al. 2012; Meigs et al. 1954; NIOSH 1986a, 1986b), and its metabolites have also been measured in the urine of volunteers exposed to 3,3'-dichlorobenzidine orally (Belman et al. 1968). In addition, 3,3’-dichlorobenzidine metabolites can form adducts with hemoglobin and DNA, and the adducts are considered to be early biological effects of 3,3-dichlorobenzidine (see Section 3.3.2). Detection of these adducts can be used as both biomarkers of exposure and biomarkers of effect. Monitoring of hemoglobin and DNA adducts combined with measuring urinary 3,3'-dichlorobenzidine and metabolite levels are effective tools for biological monitoring in humans. (Knoell et al. 2012).

3.3.2 Biomarkers of Effect

Adducts of 3,3’-dichlorobenzidine adducts with hemoglobin and DNA are considered to be an early biological effect of 3,3’-dichlorobenzidine; therefore, the detection of adducts can be used as a biomarker of effect.

In rats, the detection of hemoglobin adducts is a biomarker of exposure to 3,3'-dichlorobenzidine, indicating the detection of hemoglobin adducts as a suitable biomarker of exposure for humans. Two 3,3'-dichlorobenzidine metabolites, N-acetyl-dichlorobenzidine and N,N'-diacetyldichlorobenzidine, can form hemoglobin adducts in rats. Hemoglobin adducts have been detected in female Wistar rats orally

administered single 127 or 253 mg/kg doses of 3,3'-dichlorobenzidine (Birner et al. 1990). The investigators suggested that metabolically formed nitroso derivatives can result in the formation of a sulfinic acid amide with cysteine residues in hemoglobin (Birner et al. 1990). Hydrolysis yielded mainly 3,3'-dichlorobenzidine; N-acetylated-3,3'-dichlorobenzidine was also detected. Using a more sensitive analytical method, Joppich-Kuhn et al. (1997) also detected 3,3'-dichlorobenzidine hemoglobin adducts in rats treated repeatedly with much lower doses (0.3–5.8 mg/kg/day) of 3,3'-dichlorobenzidine in the drinking water. The limit of detection of the method was below 0.1 ng/g hemoglobin and was linear up to 150 ng/g hemoglobin.

Zwirner-Baier and Neumann (1998) analyzed hydrolysable hemoglobin adducts representing the bioavailability of N-hydroxylamines and the corresponding nitroso-derivatives following oral administration to female Wistar rats of 3,3'-dichlorobenzidine. The results showed that deamination did not take place (low adduct levels were found); the monoacetamide (N-acetyl-3,3'-dichlorobenzidine) was readily deacetylated *in vivo*, whereas the diacetamide (N,N'-diacetyl-3,3'-dichlorobenzidine) was not. In addition, acetylation polymorphism was studied with 3,3'-dichlorobenzidine in slow-acetylating A/J mice and rapid-acetylating C57BL/6J mice (Zwirner-Baier and Neumann 1998). The slow acetylator genotype was associated with significantly higher hemoglobin-adduct levels. The results provide additional support for the use of hemoglobin adducts in biomonitoring as a dosimeter for the biologically active dose of arylamines/arylacetamides.

In humans, Lee (2003) measured 3,3'-dichlorobenzidine metabolites resulting from hydrolyzing DNA adducts extracted from exfoliated bladder epithelial cells collected from the urine of workers handling 3,3'-dichlorobenzidine. Linear regressions between exposure years and DNA adduct levels were performed and found duration of employment associated with the concentration of metabolites (Lee 2003). No further human studies utilizing this biomonitoring method were located.

No disease states in humans are currently clearly associated with exposure to 3,3'-dichlorobenzidine. There is evidence that it is carcinogenic in animals (Golub et al. 1975; Osanai 1976; Pliss 1959, 1963; Stula et al. 1975, 1978) and genotoxic in test systems (Ashby and Mohammed 1988; Cihak and Vontorkova 1987; Claxton et al. 2001; Ghosal and Iba 1990; Shiraishi 1986; Wang et al. 2005). However, these effects are not unique to 3,3-dichlorobenzidine.

3.4 INTERACTIONS WITH OTHER CHEMICALS

In contrast to its effects on other mutagens and carcinogens, di-tert,-butylated hydroxytoluene (BHT) was shown to increase the mutagenicity of 3,3'-dichlorobenzidine to *Salmonella* TA98 by 21–32% and the covalent binding of 3,3'-dichlorobenzidine to added DNA by 32–76% (Ghosal and Iba 1992). BHT is an antioxidant and a free radical scavenger considered to be a cancer chemopreventative agent based on its ability to inhibit various phases of the carcinogenic process including the bioactivation and binding of carcinogenic chemical compounds to DNA (Ghosal and Iba 1992).

A synergistic role for 3,3'-dichlorobenzidine and other aromatic amines in the development of bladder cancer has been suggested. This was proposed in a study in which no carcinomas were found in any rats administered one of the following: 0.03% 3,3'-dichlorobenzidine in the diet, 0.001% BBN (N-butyl-N-(hydroxybutyl)nitrosamine) in drinking water, 0.0005% 2-AAP (2-acetylaminofluorene) in the diet, or 0.04% FANFT (N-[4-(5-nitro-2-furyl)-2thiazolyllformamide) in the diet for a period of 40 weeks (Ito et al. 1983). However, when BBN and 3,3'-dichlorobenzidine were fed together at the same dose levels as above, there was a marked increase in papillary or nodular hyperplasia in the rat bladder and the appearance of one papilloma. Based on these findings, the study authors suggested that 3,3'-dichloro-benzidine had a synergistic effect on the carcinogenicity of BBN. In rats sequentially administered BBN (0.01%), FANFT (0.15%), 2-AAF (0.025%), and 3,3'-dichlorobenzidine (0.03%) for 4 weeks, the incidence of bladder cancer after administration of the four chemicals was no different than after administration of the first three, suggesting no additive or antagonistic effect for 3,3'-dichlorobenzidine (Ito et al. 1983).

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

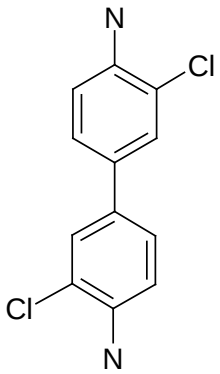
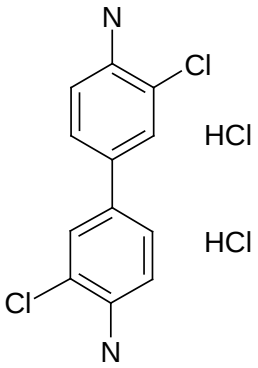
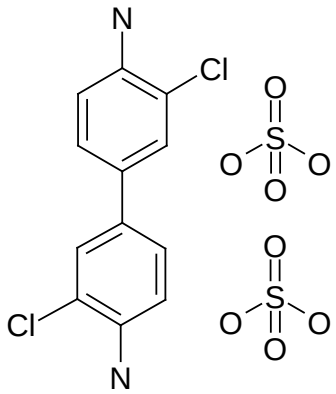
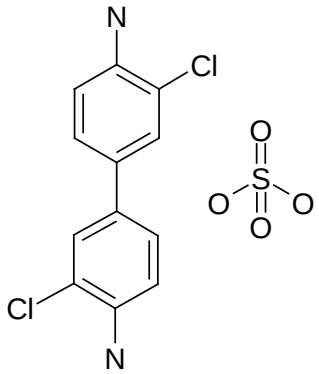
3,3'-Dichlorobenzidine is a solid crystalline powder composed of two conjoined benzene rings each with chlorine groups in the "3" positions and amino groups in the "4" positions. The primary Chemical Abstracts Service Registry Number (CASRN) for the compound includes the various salts that the base substance can form in the presence of certain compounds, especially inorganic acids. In addition to the general CASRN, 3,3'-dichlorobenzidine dihydrochloride and 3,3'-dichlorobenzidine sulfate are salts that have their own unique CASRNs, and may exhibit unique toxicological properties. 3,3'-Dichlorobenzidine is manufactured from o-nitrochlorobenzene by reduction with zinc dust and sodium hydroxide, followed by rearrangement with hydrochloric acid or sulfuric acid. Different salts are formed when 3,3'-dichlorobenzidine is exposed to certain compounds. 3,3'-Dichlorobenzidine (and its salts) was previously used in the manufacture of dyes in the United States. However, the 2016 Chemical Data Reporting (CDR) rule indicates that 3,3'-dichlorobenzidine dihydrochloride is currently used in pigment manufacturing. Information was not available on past or current uses of 3,3'-dichlorobenzidine sulfate (NLM 2019).

Table 4-1 lists common synonyms, trade names, and other pertinent identification information for 3,3'-dichlorobenzidine (and its salts), 3,3'-dichlorobenzidine dihydrochloride, and 3,3'-dichlorobenzidine sulphate, and 3,3'-dichlorobenzidine dihydrogen bis(sulphate).

4.2 PHYSICAL AND CHEMICAL PROPERTIES

3,3'-Dichlorobenzidine and its salts are solid crystalline substances. 3,3'-Dichlorobenzidine has a relatively high log K_{oc}, suggesting that it will have a low mobility in soil and will bind strongly to solid phases in soil, sediment, and sludges. The compound and its salts have relatively low (but not negligible) solubility in water. Table 4-2 lists important physical and chemical properties of 3,3'-dichlorobenzidine (and its salts), 3,3'-dichlorobenzidine dihydrochloride, 3,3'-dichlorobenzidine dihydrogen bis(sulphate), and 3,3'-dichlorobenzidine sulphate. This information includes synonyms, chemical formulas and structures, and identification numbers.

Table 4-1. Chemical Identity of 3,3'-Dichlorobenzidine (and its Salts), 3,3'-Dichlorobenzidine Dihydrochloride, 3,3'-Dichlorobenzidine Dihydrogen Bis(sulphate), and 3,3'-Dichlorobenzidine Sulphate

Characteristic	3,3’-Dichlorobenzidine (and its salts)	3,3’-Dichlorobenzidine dihydrochloride	3,3’-Dichlorobenzidine dihydrogen bis(sulphate)	3,3’-Dichlorobenzidine sulphate
Synonym(s) and Registered trade name(s)	Dichlorobenzidine; (1,1'-biphenyl)-4,4'-diamine, 3,3'-dichloro-; benzidine, 3,3'-dichloro-; DCB; 4,4'-diamino-3,3'-dichlorodiphenyl; Curithane[a]	Benzidine, 3,3'-dichloro-, dihydrochloride; (1,1'-biphenyl)-4,4'-diamine, 3,3'-dichloro-, dihydrochloride[a]	(1,1'-Biphenyl)-4,4'-diamine, 3,3'-dichloro-, sulfate (1:2)[a]	3,3'-Dichlorobenzidine sulphate; benzidine, 3,3'-dichloro-, sulfate; (1,1'-biphenyl)-4,4'-diamine, 3,3'-dichloro-, sulfate (1:1)[a]
Chemical formula	$C_{12}H_{10}Cl_2N_2$[a]	$C_{12}H_{12}Cl_4N_2$[a]	$C_{12}H_{14}Cl_2N_2O_8S_2$[a]	$C_{12}H_{12}Cl_2N_2O_4S$[a]
Chemical structure				
CAS registry number(s)	91-94-1[a]	612-83-9[a]	64969-34-2	64414-68-2 74332-73-3

[a]NLM 2019.

CAS = Chemical Abstracts Service

Table 4-2. Physical and Chemical Properties of 3,3'-Dichlorobenzidine (and its Salts), 3,3'-Dichlorobenzidine Dihydrochloride, 3,3'-Dichlorobenzidine Dihydrogen Bis(sulphate), and 3,3'-Dichlorobenzidine Dihydrogen Bis(sulphate)

Property	3,3'-Dichlorobenzidine (and its salts)	3,3'-Dichlorobenzidine dihydrochloride	3,3'-Dichlorobenzidine dihydrogen bis(sulphate)	3,3'-Dichlorobenzidine sulphate
Molecular weight	253.126 g/mol[b]	326.06 g/mol[b]	449.27 g/mol[b]	351.2 g/mol[b]
Color	White crystalline solid; gray to purple crystalline solid[b]	White crystals; white to light-gray powder; needles[b]	White crystalline powder[b]	White crystalline powder[b]
Physical state	Solid[b] (ionic species)	Solid[b] (ionic species)	Solid[b] (ionic species)	Solid[b] (ionic species)
Melting point(s)	132.5°C[b]	No data	No data	No data
Boiling point(s)	402°C[b]	No data	No data	No data
Density	Not applicable	Not applicable	Not applicable	Not applicable
Taste	No data	No data	No data	No data
Taste threshold:	No data	No data	No data	No data
Odor	No data	Mild odor[b]	No data	No data
Odor threshold:	No data	No data	No data	No data
Solubility:				
Water at 25°C	3.1 mg/L[b]	Slightly soluble in water	Slightly soluble in water	Slightly soluble in water
Organic solvent(s)	Soluble in alcohol, ether, acetic acid, and benzene; slightly soluble in hydrochloric acid[b]	Readily soluble in alcohol	No data	No data
Partition coefficients:				
Log K_{ow}	3.02–3.78[c]	No data	No data	No data
Log K_{oc}	2.86–4.67[d]	No data	No data	No data
Vapor pressure at 25°C	4.1×10^{-6} mmHg[b]	No data	No data	No data
Henry's law constant at 25°C	2.8×10^{-11} atm-m^3/mol[b]	No data	No data	No data
Dissociation constants:				
$pK_{a,1}$	1.6[a]	No data	No data	No data
$pK_{a,2}$	3.2[a]	No data	No data	No data

Table 4-2. Physical and Chemical Properties of 3,3'-Dichlorobenzidine (and its Salts), 3,3'-Dichlorobenzidine Dihydrochloride, 3,3'-Dichlorobenzidine Dihydrogen Bis(sulphate), and 3,3'-Dichlorobenzidine Dihydrogen Bis(sulphate)

Property	3,3’-Dichlorobenzidine (and its salts)	3,3’-Dichlorobenzidine dihydrochloride	3,3’-Dichlorobenzidine dihydrogen bis(sulphate)	3,3’-Dichlorobenzidine sulphate
Autoignition temperature	No data	No data	No data	No data
Flashpoint	No data	No data	No data	No data
Flammability limits in air	No data	No data	No data	No data
Conversion factors:	ppm = 0.0966 times mg/m^{3}[b]	No data	No data	No data
Explosive limits	No data	No data	No data	No data
Incompatibilities and reactivity	No data	No data	Reactivity to acidic salts and aryl halides	No data

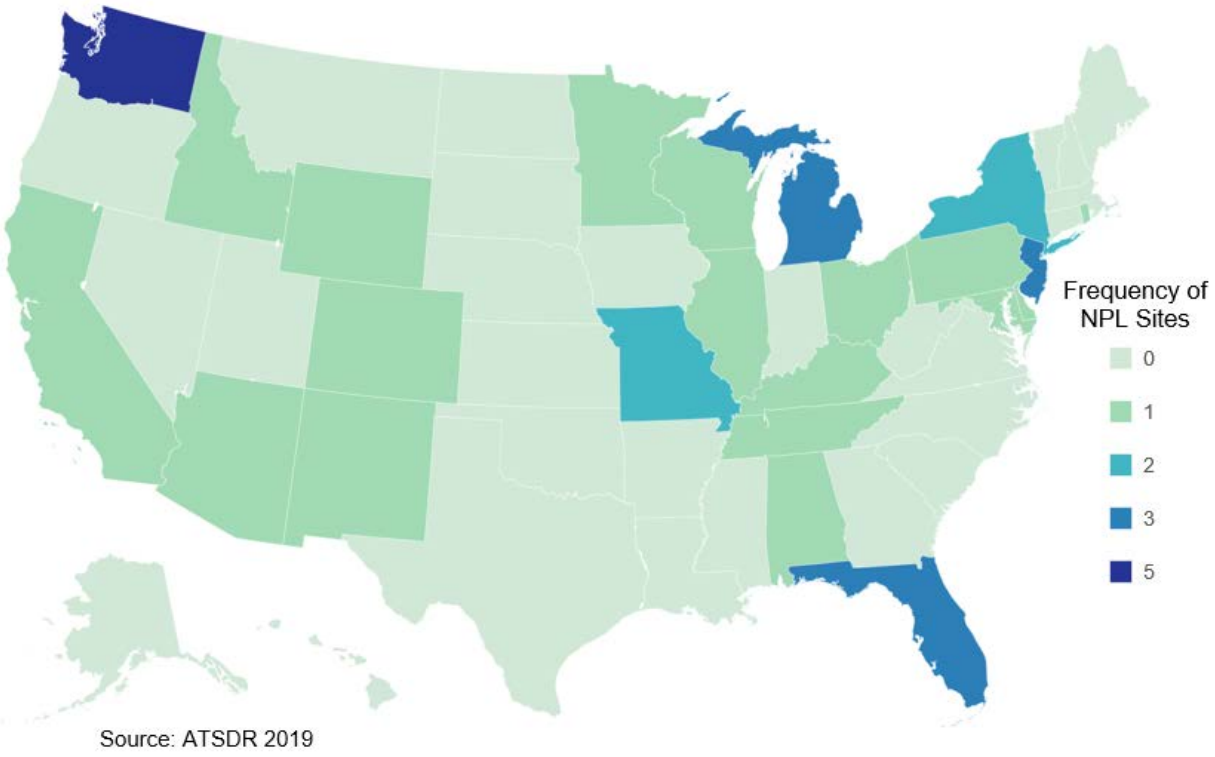
[a]Nyman et al. 1997.
[b]NLM 2019.
[c]DCMA 1989; EPA 1982.
[d]Donaldson and Nyman 2005; EPA 1982, 2014.

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

3,3'-Dichlorobenzidine has been identified in at least 35 of the 1,854 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2019). However, the number of sites evaluated for 3,3'-dichlorobenzidine is not known. The number of sites in each state is shown in Figure 5-1.

Figure 5-1. Number of NPL Sites with 3,3'-Dichlorobenzidine Contamination



- Exposure to 3,3'-dichlorobenzidine primarily occurs in occupational settings by inhalation and dermal exposure in industries that manufacture or use the chemical.
- Communities near dye manufacturers, or hazardous waste sites, are most likely to be exposed to industrial wastewater effluents containing 3,3'-dichlorobenzidine.
- Human exposure can also occur from use of personal care products containing small amounts of 3,3'-dichlorobenzidine and through ingestion of paint chips containing 3,3'-dichlorobenzidine as a pigment.

- 3,3'-Dichlorobenzidine is commercially produced for industrial use as a dye or pigment, primarily in ink, textile, rubber, and plastics industries. It enters the environment through industrial wastewater. It is not naturally occurring.

- The manufacturing of 3,3'-dichlorobenzidine has markedly decreased since 1986 in the United States, but large quantities are still imported into the United States and occupational exposure remains the major source of exposure.

- In the environment, 3,3'-dichlorobenzidine is primarily found in soils and sediments since it binds to sediments and has low mobility.

- Photodegradation is an important fate process in air, water, soil, and sediment, has been observed in controlled laboratory settings, and is expected to occur in the environment under natural sunlight.

5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.2.1 Production

3,3'-Dichlorobenzidine is a chlorinated primary aromatic amine (NTP 2016; WHO 1998). The primary anthropogenic source of 3,3'-dichlorobenzidine is pigments and dyes. 3,3'-Dichlorobenzidine is no longer used to manufacture dyes in the United States (CPMA 1998). 3,3'-Dichlorobenzidine is commercially produced by reduction of o-nitrochlorobenzene with zinc dust and sodium hydroxide solution, and the resulting hydrazobenzene derivative is rearranged with dilute hydrochloric acid or sulfuric acid to form 3,3'-dichlorobenzidine (DCMA 1989; Schwenecke and Mayer 2000). Commercial supplies are usually provided in the form of the dihydrochloride salt because of its greater stability.

In 1986, there were approximately 10 suppliers of the chemical listed in the United States (NTP 1991). 3,3'-Dichlorobenzidine was produced by 1 manufacturer in Europe in 2009; the hydrochloride was produced by 10 manufacturers (1 in Europe, 1 in China, 2 in East Asia, and 6 in India). There were 14 suppliers of 3,3'-dichlorobenzidine worldwide, including 8 in the United States (NTP 2016). The 2016 CDR reported the national aggregate production volume of the 3,3'-dichlorobenzidine dihydrochloride salt to be between 1,000,000 and 10,000,000 pounds in 2012, 2013, 2014, and 2015 (CDR 2016).

No information regarding facilities that produce, process or use 3,3'-dichlorobenzidine was reported to the EPA's Toxic Release Inventory (TRI) Program (TRI20 2021).

5.2.2 Import/Export

According to the 2016 CDR, Flint Group in Plymouth, Michigan; Sumitomo Corporation of Americas in New York, New York; and Sun Chemical in Parsippany, New Jersey manufacture or import 3,3'-dichlorobenzidine dihydrochloride (CDR 2016). Activity at Sun Chemical is confidential, but Flint Group and Sumitomo report that the dihydrochloride is imported, although production volume for all three sites is withheld (CDR 2016). Imports of the dihydrochloride amounted to 5,773,111 pounds (2,618,639 kg) in 2018, and came from India and China (USITC 2021).

5.2.3 Use

3,3’-Dichlorobenzidine had been primarily used in the production of yellow, and some red and orange, pigments for the textile, paint, printing inks, paper, rubber, plastic, and pharmaceutical industries (EPA 2010; NLM 2019). Currently, use of 3,3'-dichlorobenzidine in the production of dyes has stopped, as dye production in the United States has largely ceased and has shifted abroad (Dapson 2009). 3,3'-Dichlorobenzidine has also been used as a curing agent for isocyanate-containing polymers and solid urethane plastics, and as a compounding ingredient for rubber and plastics (NLM 2019). The chemical can also be used to produce polybenzimidazole (Maner et al. 2009) or as a color test for the detection of gold (NLM 2019). 3,3'-Dichlorobenzidine has been detected in the wastewater of metal finishing operation facilities and oilfield operations (see Section 5.5), suggesting that it is used in these industries, but no specific information on its use is available. Additionally, the chemical is likely used in steam electric power generation based on its EPA regulatory limitation (EPA 2018a).

5.2.4 Disposal

Facilities that generate 3,3'-dichlorobenzidine-containing wastes, and owners and operators of hazardous waste treatment, storage, and disposal facilities, must also comply with regulations promulgated under the authority of the Resource Conservation and Recovery Act (RCRA) (EPA 2011, 2015).

Disposal of wastes containing 3,3'-dichlorobenzidine is controlled by a number of federal regulations (see Chapter 7). The standard treatment technologies specified for treating 3,3'-dichlorobenzidine-containing wastewaters prior to land disposal are wet air oxidization, chemical or electrolytic oxidation, carbon absorption, and incineration; for non-wastewater treatment, only incineration (EPA 2003).

A 1986 health hazard evaluation report was conducted by the National Institute for Occupational Safety and Health (NIOSH) on a company that purchased 3,3'-dichlorobenzidine as the dihydrochloride salt in sealed fiber in drums. The report stated the company rinsed the empty drums with water, added the rinse water to the product stream, then sprayed the drums with a sodium hypochlorite bleach solution (converting the 3,3'-dichlorobenzidine to a less toxic quinone-type compound), and placed them in polyethylene bags for disposal (NIOSH 1986b).

5.3 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ ≥10 full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005).

5.3.1 Air

No information regarding releases of 3,3’-dichlorobenzidine was reported to the U.S. EPA’s TRI Program (TRI20 2021).

5.3.2 Water

There is no information on releases of 3,3'-dichlorobenzidine to water from manufacturing and processing facilities because these releases are not required to be reported (EPA 2005).

The free base form of 3,3'-dichlorobenzidine is only slightly soluble in water, and will rapidly adsorb and bind to sediment and particulate matter (NTP 2016). It may also undergo photolysis in water exposed to sunlight. The solubility of 3,3'-dichlorobenzidine-2HCl in water is 4 mg/L at a pH of 6.9 (Banerjee et al. 1978). 3,3'-Dichlorobenzidine is primarily released into municipal sanitary sewer systems in wastewater generated by the production of dyes and pigments, including from tanneries (Cao et al. 2007; Lee et al. 2004). The EPA measured maximum 3,3'-dichlorobenzidine concentrations in wastewater of 0.07 ppb in metal finishing operations, 2 ppb in nonferrous metals manufacturing, 3 ppb in coal mining, and up to 10 ppb in paint and ink formulation operations (EPA 1980b).

The Massachusetts Water Resource Authority (MWRA) measured average influent concentrations of 3,3’-dichlorobenzidine of 2.13–2.25 µg/L, and average effluent concentrations of 2.18–2.28 µg/L, in two wastewater treatment plants (MWRA 2015).

5.3.3 Soil

There is no information on releases of 3,3'-dichlorobenzidine to soil from manufacturing and processing facilities because these releases are not required to be reported (EPA 2005).

3,3'-Dichlorobenzidine binds to soil, and volatilization from most soil surfaces is not expected (NLM 2019). Industrial wastewater entering aqueous environments contributes to the presence of 3,3'-dichlorobenzidine in soils, as it has been detected in surface water sediments (Nyman et al. 2004). No quantitative data could be located measuring environmental releases or off-sites transfers of 3,3'-dichlorobenzidine to soil or sediment.

5.4 ENVIRONMENTAL FATE

5.4.1 Transport and Partitioning

Air. 3,3'-Dichlorobenzidine is not a volatile chemical and has a low vapor pressure and Henry’s law constant (see Table 4-2), suggesting that the atmosphere is not important in its environmental transport and partitioning (EPA 2015). Any 3,3'-dichlorobenzidine released to the air will adsorb to airborne dust particles or bind to particulate matter (NTP 2016). Suspended 3,3'-dichlorobenzidine is subject to atmospheric convection, dispersion, gravitational settling, and wash-out by rain. Particulate-phase 3,3'-dichlorobenzidine may be removed by wet and dry deposition (NLM 2019).

Water. 3,3'-Dichlorobenzidine has low solubility in water, does not volatilize or hydrolyze, and may slowly oxidize in solution (Banerjee et al. 1978; EPA 1979, 1982). The Henry's law constant for a compound helps estimate the partitioning of the compound between its vapor phase and aqueous media. The estimated Henry's law constant for 3,3'-dichlorobenzidine of $2.8x10^{-11}$atm-m^3/mole suggests that 3,3'-dichlorobenzidine remains dissolved in water and does not volatize from water to air (NLM 2019).

Use of Fenton's reagent (Fe(II) + H_2O_2) for treatment of wastes contaminated with various carcinogenic aromatic amines was investigated by Casero et al. (1997). Under laboratory conditions, 3,3'-dichloro-benzidine was converted to ring-cleavage products, with 99.9% efficiency after 1 hour of treatment at room temperature, demonstrating that this technology is potentially useful as a wastewater treatment technique (Casero et al. 1997).

Sediment and Soil. 3,3'-Dichlorobenzidine is moderately hydrophobic, and will bind to soil and sediments (Nyman et al. 1997). The chemical can exist as a weak base in water and exists in both neutral and cationic forms. Written as an acid-base reaction, the amine groups may be protonated as follows:

$3,3'\text{-DCB} + H_2O \leftrightarrow 3,3'\text{-DCBH}^+ + OH^-$

$3,3'\text{-DCBH}^+, H_2O \leftrightarrow 3,3'\text{-DCBH}_2^{2+} + OH^-$

Values of pK_a reported for 3,3'-dichlorobenzidine vary. EPA (1978b) reported a pK_a of <4, and Nyman et al. (1997) reported $pK_{a,1}$ and $pK_{a,2}$ values of 1.6 and 3.2, respectively. This indicates that the dominant state of 3,3'-dichlorobenzidine in water would be the non-ionic form. As pH increases, the proportion of cationic forms of 3,3'-dichlorobenzidine decreases. The extent of adsorption to sediments via Coulombic interactions will thus decrease, and adsorption should become dominated by hydrophobic interactions as pH increases. This expectation was demonstrated by EPA (1978b), who found that the adsorption constant (K_f) decreased with increasing pH; the decrease was more rapid in the range of pH 7–9. Boyd et al. (1984) concluded that non-protonated 3,3'-dichlorobenzidine is subject to hydrophobic bonding to some extent. However, no correlation has been found between K_f and the organic carbon content of sediment and soil (Boyd et al. 1984; EPA 1978b; Graveel et al. 1986).

Aromatic amines are also expected to bind to soils through irreversible covalent bonding, especially to soils containing large organic carbon content (Donaldson and Nyman 2005). 3,3'-Dichlorobenzidine covalently bonds with soil humic and fulvic components (Boyd et al. 1984; EPA 1978b; NLM 2019), and

these bonds are often irreversible and immobile. Studies show that the extent of 3,3'-dichlorobenzidine desorption decreases with age of the sample, and that the adsorbed 3,3'-dichlorobenzidine is resistant to extraction. After 24 hours of 3,3'-dichlorobenzidine-sediment contact, only 36% of the parent compound could be extracted by methanol (Boyd et al. 1984; EPA 1978b). A result of this complex behavior is that adsorption constants (K_f) for 3,3'-dichlorobenzidine cannot be accurately predicted for a given soil based on a K_{oc} value alone.

K_{oc} values measured for 3,3'-dichlorobenzidine range from 721 to 47,000. The EPA's TRI Program reported a K_{oc} value of 47,000 (EPA 2014); K_{oc} values ranging from 721 to 3,965 were measured in sediment samples from Lake Macatawa in Michigan (Donaldson and Nyman 2005); EPA (1982) reported a K_{oc} value of 1,553. These relatively high values imply that 3,3'-dichlorobenzidine would exhibit low to no mobility in soil (see Roy and Griffin 1985). 3,3'-Dichlorobenzidine was highly immobile in soil column experiments (Chung and Boyd 1987). Water was passed through sandy soil (Entic Haplorthod) and 3,3'-dichlorobenzidine-contaminated sewage sludge samples. Only small amounts of radioactive 3,3'-dichlorobenzidine added to columns of sandy soil or sewage sludge were eluted with water over extended time periods. Extractable radioactivity from these soils and sludge samples decreased with time of chemical contact. There was greater adsorption of 3,3'-dichlorobenzidine to soil than to sludge as a result of the greater humus content of the soil samples, suggesting that the compound may favor migration from sludge to soil substrates (Chung and Boyd 1987).

3,3'-Dichlorobenzidine bound to sediment can be transported over relatively long distances, attributed to its hydrophobicity (Harden et al. 2005). An 11-year field study measured higher levels of 3,3'-dichlorobenzidine 6 km from its known source in Lake Macatawa, Michigan, than in the sediment immediately adjacent to that source. This transport likely occurred due to sediment resuspension events in the lake (Harden et al. 2005).

The Henry's law constant of 3,3'-dichlorobenzidine indicates that it is not expected to volatilize from moist soil surfaces. Volatilization from dry soil surfaces is also not expected due to the chemical's low vapor pressure (EPA 2015). Boyd et al. (1984) found no loss of 3,3'-dichlorobenzidine from soil due to volatilization during 32- and 52-week studies under aerobic and anaerobic conditions, respectively.

Other Media. Since 3,3'-dichlorobenzidine is moderately hydrophobic (see Table 4-2), it may be concentrated from aqueous media by aquatic organisms, and some bioaccumulation in aquatic organisms may occur (Law 1995). Bluegill sunfish were exposed to radiolabeled 3,3'-dichlorobenzidine in dynamic-

flow experiments for 130–168 hours, and transferred to clean water free of the chemical (Appleton and Sikka 1980). The chemical and its metabolites showed some accumulation. They were not completely eliminated upon transfer, as residues remained after 14 days. Appleton and Sikka (1980) calculated moderately low bioconcentration factors (BCFs) of 495–507 for whole fish. The edible and non-edible portions of the fish had BCFs of 114–175 and 814–856, respectively. BCFs for golden ide fish of 610 and for green algae of 940 have been reported (Freitag et al. 1985). A BCF range of 43–213 was calculated for carp exposed to 3,3'-dichlorobenzidine over an 8-week period (NLM 2019). These BCF data suggest that the potential for bioconcentration in aquatic organisms is low to moderate.

Bioaccumulation by terrestrial animals has not been studied. Assuming a log K_{ow} range of 3.02–3.78 (DCMA 1989; EPA 1982), 3,3'-dichlorobenzidine is not likely to bioaccumulate in plants or terrestrial animals appreciably.

A BCF was estimated for 3,3'-dichlorobenzidine in fruits (oranges) of 1.77 L kg^{-1} (Paraiba et al. 2006). The study applied Fruit Tree Model (FTM) to a hypothetical culture of orange orchards, and the BCF estimated by the model was considered applicable to other fruits. The study examined fruit from orange orchards cultivated in soils treated with sludge originating from sewage treatment plants (Paraiba et al. 2006).

5.4.2 Transformation and Degradation

Air. 3,3'-Dichlorobenzidine in sunlight in ambient air may react with photochemically-produced hydroxyl radicals and ozone with an estimated half-life of 10 hours (NLM 2019). This was calculated from an EPA-estimated hydroxyl radical reaction rate constant of $4x10^{-11}$ cm^3/molecule-second at 25°C (EPA 2015). No other information on the fate of 3,3'-dichlorobenzidine in the atmosphere was located.

Water. 3,3'-Dichlorobenzidine was found to be extremely photolabile in water and is expected to be susceptible to photolysis in sunlit surface water, an important fate process (Banerjee et al. 1978; EPA 1978b). When exposed to laser radiation at wavelengths ranging from 300 to 360 nm in aqueous solutions, 3,3'-dichlorobenzidine photodechlorinated (Nyman et al. 2002). Chlorobenzidine was observed as an unstable intermediate, yielding benzidine as a stable photoproduct. Photochemical half-lives were calculated and ranged from 279 to 3,013 seconds for 3,3'-dichlorobenzidine (Nyman et al. 2002). When exposed to artificial light, 3,3'-dichlorobenzidine photolyzed yielding monochlorobenzidine, benzidine,

and a number of colored, water-insoluble products. In natural sunlight, the half-life of 3,3'-dichlorobenzidine in water was determined to be approximately 90 seconds. While 3,3'-dichlorobenzidine is very rapidly photolyzed under environmental conditions, the process may yield benzidine (Banerjee et al. 1978).

Biodegradation of 3,3'-dichlorobenzidine is slow in water. When incubated in natural water from eutrophic and mesotropic lakes, 25% of the chemical biodegraded within a month (Boyd et al. 1984).

The composition of the biological community was not described. Minor decreases in 3,3'-dichlorobenzidine concentrations were attributed to adsorption onto suspended sediment. Half-lives of 4–26 weeks and 16–101 weeks have been estimated for 3,3'-dichlorobenzidine biodegradation in surface water and anaerobic groundwater, respectively (Howard et al. 1991).

There are no data to suggest that the hydrolysis of 3,3'-dichlorobenzidine is significant (EPA 1979). In one study, no hydrolysis of 3,3'-dichlorobenzidine was observed after 5 days at elevated temperature (50°C) (NLM 2019). A half-life of 100 days was estimated based on surrogate substances (NLM 2019).

Several studies (Mizuno et al. 2007; Ohe et al. 2008) reported 3,3'-dichlorobezidine contamination of the Waka River in Japan. Mizuno et al. (2007) detected high levels of 3,3'-dichlorobenzidine and 4-amino 3,3'-dichloro-5,4-dinitrobiphenyl (ADDB), a mutagenic compound and endocrine disruptor, from samples collected at chemical plants' wastewater discharge into the river. ADDB is formed by oxidation and nitration from 3,3'-dichlorobenzidine during the process of wastewater treatment of drainage. Mutagenicity of water samples was evaluated in the *Salmonella* assay using the O-acetyltransferase-overexpressing strain YG1024. Water samples from the discharge site showed stronger mutagenicity than water samples collected from upstream and downstream sites. Similarly, Ohe et al. (2008) identified two mutagenic fractions (YG1024 strain) in the water adsorbate, 3,3'-dichlorobenzidine, and a novel chemical, a 5-nitro derivative of 3,3'-dichlorobenzidine (4,4'-diamino-3,3'-dichloro-5-nitrobiphenyl), thought to be formed from 3,3'-dichlorobenzidine during the wastewater treatment process.

Sediment and Soil. Sediment/water mixtures spiked with 3,3'-dichlorobenzidine display evidence of the chemical's degradation (Nyman et al. 1997). Silty-clay to sandy sediments collected from a lake near Holland, Michigan, were spiked with 3,3'-dichlorobenzidine and incubated at 20°C for 12 months under anaerobic conditions. Time-course analysis of this mixture showed that dehalogenation of 3,3'-dichloro-

benzidine to produce benzidine appears to take place through a transient intermediate, 3-monochloro-benzidine. Up to 80% of the 3,3'-dichlorobenzidine was transformed to benzidine over a 1-year incubation period. No metabolites were observed in autoclaved samples, suggesting that dehalogenation is mediated by microbial activity. The final product, benzidine, shows more affinity for the solution (aqueous) phase and thus has a greater potential for transport in the environment. A half-life of approximately 150 days was estimated for 3,3'-dichlorobenzidine in lake water and sediment mixes (Nyman et al. 1997).

The Japanese MITI test classified 3,3'-dichlorobenzidine as not readily biodegradable (NLM 2019). The chemical (at a concentration of 100 mg/L) was found to achieve 1% of its theoretical biological oxygen demand (BOD) in 4 weeks (NLM 2019). A summary of seven laboratory tests conducting aerobic biodegradation experiments with 3,3'-dichlorobenzidine concluded that while results showed its “inherent biodegradability,” the compound should not be classified as readily biodegradable (Brown and Laboureur 1983). There was a clear dependence of the extent of degradation on the concentration of yeast extract added to the batch containers. The role of the extract was uncertain, but without it, no degradation was detected. The study authors hypothesized that the yeast may be a food source to allow buildup of large concentrations of active bacteria that are able to break down the amines. Possible degradation mechanisms and degradation byproducts were not discussed (Brown and Laboureur 1983).

Laboratory experiments designed to probe biodegradation and photodegradation pathways showed that 3,3'-dichlorobenzidine undergoes sequential dehalogenation to yield 3-chlorobenzidine and then benzidine under exposure to microorganisms and under simulated tropospheric solar radiation (Nyman et al. 1999). Dechlorination is expected to yield higher total concentrations of aromatic amines in the solution (Nyman et al. 1999).

3,3'-Dichlorobenzidine degraded very little when incubated with soil. In a study by Boyd et al. (1984), a Brookston clay loam soil (a typic Argiaquoll fine loamy, mixed mesic) containing [^{14}C]-3,3'-dichloro-benzidine at concentrations of 40 and 4 mg/kg of dry soil was incubated aerobically and anaerobically in batch experiments (Boyd et al. 1984). Under aerobic conditions, 3,3'-dichlorobenzidine degradation occurred at a very slow rate; cumulative $^{14}CO_2$ production was approximately 2% after 32 weeks. Under anaerobic conditions, no gas evolution was detected after 1 year of incubation. The authors did not comment on the population or type of microorganisms in the soil sample (Boyd et al. 1984). Additional studies indicated that 3,3'-dichlorobenzidine was very persistent in soil and sludge-amended soil (Chung and Boyd 1987). Biodegradation of [^{14}C]-3,3'-dichlorobenzidine was evaluated over a 182-day

incubation period in a sandy soil (Entic Haplorthod) amended with sewage sludge. The total amount of [^{14}C]-3,3'-dichlorobenzidine recovered as $^{14}CO_2$ was <2%. It should be noted that biodegradation when measured by $^{14}CO_2$ evolution may provide a conservative estimate of the extent of decomposition. This technique does not account for carbon that is incorporated into the biomass or into soil organic matter, or for the compound being only partially metabolized (Graveel et al. 1986). The disparity between the results of this work and the results of Nyman et al. (1997) was likely related to the nature of their respective biotic communities.

As previously discussed, in aqueous solutions 3,3'-dichlorobenzidine rapidly undergoes photolysis and strong absorption of light at relatively high wavelengths. This suggests that 3,3'-dichlorobenzidine may be susceptible to direct photolysis on soil surfaces exposed to natural sunlight (NLM 2019). Benzidine, as a product of 3,3'-dichlorobenzidine photolysis, has been found in sediment testing for 3,3'-dichlorobenzidine (Harden et al. 2005). The study found that environmental transformation to benzidine in the natural environment is of possible concern. No further information was located on this suggested transformation.

5.5 LEVELS IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to 3,3'-dichlorobenzidine depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of 3,3'-dichlorobenzidine in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on 3,3'-dichloro-benzidine levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

Table 5-1 shows the limit of detections typically achieved by analytical analysis of environmental media. 3,3'-Dichlorobenzidine has been detected in varying environmental media and products. An overview summary of the range of concentrations detected in environmental media is presented in Table 5-2.

Table 5-1. Lowest Limit of Detection for 3,3'-Dichlorobenzidine Based on Standards

Media	Detection limit	Reference
Air	0.05 µg/sample	NIOSH 2020
Wastewater	0.01 µg/L	Lee et al. 2004
Surface water	0.05 µg/L	Vera-Avila et al. 2001
Drinking water	0.2 µg/L	Onuska et al. 2000
Sediment	15 µg/L	Armentrout and Cutie 1980; Harden et al. 2005
Fish tissue	<20 µg/L	Diachenko 1979
Human hemoglobin adducts	<0.1 ng/g	Joppich-Kuhn et al. 1997
Rat hemoglobin adducts	0.01 µg/L	Lee et al. 2003
Urine (dichlorobenzidine, mono- and di-acetyldichlorobenzidine)	525 to 600 µg/L	Bowman and Nony 1981; Nony and Bowman 1980; Nony et al. 1980
Urine	1.6 µg/L	Guerbet et al. 2007
Leather	<1 mg/kg	Sparr Eskilsson et al. 2002
Commercial dyestuff	0.9 µg/g	Wu and Huang 1998

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

Table 5-2. Summary of Environmental Levels of 3,3'-Dichlorobenzidine in the United States

Media	Low	High	For more information
Sediment (ppm)	Not detected	69.663	Section 5.5.3
Indoor air (ppbv)	Not detected	ND	Section 5.5.1
Surface water (ppm)	Not detected	26.8	Section 5.5.2
Groundwater (ppm)	<10	0.90	Section 5.5.2
Pore water (ppm)	6.53	8.71	Section 5.5.2
Soil	Not detected	Not detected	Section 5.5.3

Table 5-3 shows the levels of 3,3'-dichlorobenzidine in environmental media at NPL sites.

Table 5-3. 3,3'-Dichlorobenzidine Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measures	NPL sites
Water (ppb)	8.00	22.4	9.03	3	2
Soil (ppb)	5,080	7,070	8.77	10	7
Air (ppbv)	NA	NA	NA	NA	NA

[a]Concentrations found in ATSDR site documents from 1981 to 2019 for 1,854 NPL sites (ATSDR 2019). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

5.5.1 Air

3,3'-Dichlorobenzidine was not detected in ambient air of two dyestuff production plants at detection limits of 5 (Narang et al. 1982) and 0.1 ng/m^3 (Riggin et al. 1983). Data on occupational exposure levels indicate the presence of levels ≤0.6–2.5 μg/m^3 in 3,3'-dichlorobenzidine production and pigment manufacturing plants in Germany (DCMA 1989).

The concentration of 3,3'-dichlorobenzidine in the Canadian environment was estimated by Liteplo and Meek (1994) by applying the Level III Fugacity Computer Model of Mackay and Paterson (Mackay and Paterson 1991). The model assumed that 1% of the total amount produced in and imported to Canada was released into various media in proportions similar to those given in the U.S. TRI. The average concentration of 3,3'-dichlorobenzidine in air, as estimated by the model, was $7.6x10^{-16}$ μg/m^3 (Liteplo and Meek 1994).

5.5.2 Water

EPA's computerized water quality database (STORET) was used to determine the median concentration of 3,3'-dichlorobenzidine in surface water, groundwater, and municipal and industrial inflow and outflow (Staples et al. 1985). The median concentration of 3,3'-dichlorobenzidine detected in 12 of 1,239 samples of waste effluent, collected from about 1980 to 1984, was reported to be <10 μg/L. The median concentration of 3,3'-dichlorobenzidine in both surface water and groundwater was also reported to be

<10 µg/L. The U.S. Geologic Survey measured 3,3'-dichlorobenzidine concentrations of 0.65–0.90 µg/L groundwater from wells of an oilfield service site in Oklahoma from 2005 to 2006 (USGS 2007).

EPA indicated that 3,3'-dichlorobenzidine concentrations in wastewater from metal finishing operations were ≤0.07 µg/L (EPA 1980b). Discharge concentrations from other industrial sources were ≤10 µg/L. Using a Fugacity Computer Model, Liteplo and Meek estimated the concentration of 3,3'-dichlorobenzidine in Canadian water to be $3.4x10^{-7}$ ng/L (Liteplo and Meek 1994). Because the model did not address the possibility of bound residue in sediment, the concentration in water is likely overestimated.

Onuska et al. (2000) extracted 3,3'-dichlorobenzidine from industrial effluent samples close to an industrial pigment site in Toronto, Canada. Water concentrations near the Toronto pigment site ranged from 2.60 to 654 µg/L. Lee et al. (2004) followed up testing at this site, and measured concentrations as low as 1.6 µg/L in 2001. This significant decrease from the 1996 concentrations measured by Onuska et al. (2000) (>600 µg/L) was attributed to stringent pollution control programs adopted by the company (Lee et al. 2004). Wastewater effluent from four metal plating companies showed that two companies had non-detectable amounts of 3,3'-dichlorobenzidine, and two companies had detectable trace levels of 3,3'-dichlorobenzidine of 0.013 and 0.032 µg/L (Lee et al. 2004). Non-detectable amounts of 3,3'-dichlorobenzidine were detected from the other two companies.

Harden et al. (2005) detected up to 26.8 µg/L of 3,3'-dichlorobenzidine in lake water and 6.53–8.71 µg/L in sediment pore water. 3,3'-Dichlorobenzidine concentrations tended to be higher in the pore water of silty clay sediments.

Capillary gas chromatography/mass spectrometry (GC/MS) was used to identify, but not quantify, 3,3'-dichlorobenzidine in the dissolved phase (that is, smaller particles and dispersed colloids not retained by the filter) of water concentrates from the Besos River in Spain (Grifoll et al. 1992). Valls et al. (1990) identified 3,3'-dichlorobenzidine in urban wastewater in the same region.

5.5.3 Sediment and Soil

The estimated median concentration of 3,3'-dichlorobenzidine in sediments in the United States has been reported to be <1 µg/kg on a dry sediment basis (Staples et al. 1985). Of the 347 sediment or soil measurements recorded in the STORET database, none of the samples contained detectable concentrations of 3,3'-dichlorobenzidine.

Harden et al. (2005) measured concentrations of 3,3'-dichlorobenzidine from nondetectable up to 69.663 mg/kg-sediment near Lake Macatawa from 1993 to 2003. The highest level was found 6 km away from the single known source of 3,3'-dichlorobenzidine in the area (Harden et al. 2005). This finding was supported by the oscillatory pattern of 3,3'-dichlorobenzidine distribution within Lake Macatawa where sampling sites of nondetectable 3,3'-dichlorobenzidine concentrations were adjacent to sites of high concentrations. The pattern was explained by sediment resuspension and transport mostly due to wind-driven resonant motions (Nyman et al. 2003). 3,3'-Dichlorobenzidine levels of 0.01–0.04 mg/kg were detected in Canadian agricultural soils (NLM 2019).

5.5.4 Other Media

The estimated median concentration of 3,3'-dichlorobenzidine in biota in the United States has been reported to be <2.5 mg/kg wet (Staples et al. 1985). Of the 83 biota measurements recorded in the STORET database, none of the samples contained detectable concentrations of 3,3'-dichlorobenzidine.

There is a potential for 3,3'-dichlorobenzidine to occur in wastewater sludges and industrial solid wastes. A 3,3'-dichlorobenzidine concentration of 16 ppm in municipal sludge from Michigan has been reported (Chung and Boyd 1987). 3,3'-Dichlorobenzidine was detected at concentrations of 3.13 mg/kg dry sewage sludge in two of a total of 253 sewage treatment plants examined (Fricke et al. 1985). These plants were all in the United States (Arizona, Indiana, Michigan, Missouri, New Mexico, New York, and Texas). Concentrations up to 535 µg/L were detected in a communal sewage treatment plant (Lopez-Avila et al. 1981). The chemical was detected at 8.55 mg/kg in sewage sludge of an aeration basin in Muskegon, Michigan (Demirjian et al. 1984).

BCFs for 3,3'-dichlorobenzidine were measured for orange orchards cultivated in soils treated with sludge originating from sewage treatment plants (Paraiba et al. 2006). The study did not provide the concentration of 3,3'-dichlorobenzidine found in soil or orange orchards, but suggests that 3,3'-dichlorobenzidine can occur in produce grown in contaminated soils (Paraiba et al. 2006).

[^{14}C]-3,3'-Dichlorobenzidine was found to rapidly accumulate in bluegill sunfish as a result of their exposure to water in which either 5 or 100 µg/L of the chemical was intentionally added. Residues were

distributed in both the edible and nonedible portions (Appleton and Sikka 1980). However, 3,3'-dichlorobenzidine was not detected in fish samples obtained from rivers near nine textile dyestuff manufacturers known to use 3,3'-dichlorobenzidine-based pigments (Diachenko 1979).

In cosmetic products, 3,3'-dichlorobenzidine has been detected in concentrations as low as 0.14 mg/kg in skin oils, and as high as 2.05 mg/kg in hair gels (Hailong et al. 2014). Cosmetic samples were taken from commercial products in China, and 3,3'-dichlorobenzidine was also detected in trace amounts in hair dye, talcum powder, facial skin care mask, and facial cream. The study did not comment on possible dermal absorption to humans but suggests that 3,3'-dichlorobenzidine and other aromatic amines can be found in trace amounts in consumer cosmetic products (Hailong et al. 2014).

5.6 GENERAL POPULATION EXPOSURE

The greatest chance of exposure to 3,3'-dichlorobenzidine by the general public is from its persistence in the environment attributed to improper land disposal of compounds. The importance of this exposure source can only be evaluated on a site-by-site basis, but the potential for nonindustrial exposure via air, soil, or water is expected to be negligible. 3,3'-Dichlorobenzidine is no longer used to manufacture soluble dyes in the United States (CPMA 1998). Previously benzidine and its congeners such as 3,3'-dichlorobenzidine were likely to only be found in the vicinity of pigment plants (EPA 1978a, 1980a, 1980b) where wastes may escape or be discharged. 3,3'-Dichlorobenzidine was also found in locations where it was used to formulate other products such as rubber and plastic (NLM 2019).

In the past, the general public may have been exposed to minute amounts of 3,3'-dichlorobenzidine during the use of pressurized spray containers of paints, lacquers, and enamels containing traces of benzidine yellow, a pigment derived from 3,3'-dichlorobenzidine (EPA 1978a). Exposure of the general population, primarily children, may occur through ingestion of paint chips containing 3,3'-dichlorobenzidine as a pigment.

3,3'-Dichlorobenzidine-based pigments have been used in printing ink applications; their use in paints is rare and, thus, its presence in present-day pressurized paint spray would not be expected (CPMA 1998).

Trace amounts of 3,3'-dichlorobenzidine have been detected in various consumer cosmetics products sold abroad, including facial skin care masks, skin oils, talcum powder, hair gel, and hair dye (Hailong et al. 2014). While 3,3'-dichlorobenzidine is not directly used in the production of cosmetic products, the

decomposition of azo dyes used in cosmetic colorants can result in the presence of aromatic amines (Hailong et al. 2014).

3,3'-Dichlorobenzidine has been identified as a degradation product in pigments, commonly used in tattooing, exposed to sunlight and laser irradiation (Hauri and Hohl 2015). Irradiation by laser of C.I. Yellow Pigment 14, C.I. Yellow Pigment 83, and C.I. Orange Pigment 13 resulted in 3,3'-dichlorobenzidine as a degradation product. Irradiation by different sunlight sources of C.I. Yellow Pigment 14, C.I. Orange Pigment 13, and C.I. Orange Pigment 34 also resulted in 3,3'-dichlorobenzidine as a degradation product (Hauri and Hohl 2015). These results suggest that skin tattoos using pigments containing azo dyes may yield 3,3'-dichlorobenzidine.

Occupational exposure to 3,3'-dichlorobenzidine is most likely to occur in the synthesis of pigments, the compounding of lead iodide, and the garment, leather, printing, paper, and homecraft industries where benzidine-based pigments are used. Since 1974, Occupational Safety and Health Administration (OSHA) regulations have set strict standards for worker protection, required the use of closed manufacturing vessels, and prescribed methods to chemically destroy residues. Nevertheless, 3,3'-dichlorobenzidine has been detected in urinary samples collected from workers of facilities where the chemical had been used. Less than 0.2 μg/L of 3,3'-dichlorobenzidine was detected in urine samples of 36 workers exposed to pigments derived from the compound (Hatfield et al. 1982). 3,3'-Dichlorobenzidine levels of 0.006–0.281 ppm were measured in the urine of workers of a manufacturing plant producing the chemical, and in some of their family members (ATSDR 1996).

In Canada, the estimated daily intake of 3,3'-dichlorobenzidine by various segments of the population has been calculated. The calculations are based on the predicted levels of 3,3'-dichlorobenzidine in air, water, and soil, as well as on the estimated daily intake of each (air, water, soil) by Canadians (Government of Canada 1993). The predicted concentrations or human intake levels were not measured values but rather predicted values based on output from mathematical models using worst-case assumptions that did not take into consideration removal mechanisms such as photolysis, oxidation, or irreversible binding to substrates. The total intake by adults (≥20 years of age) was predicted to be 7.4×10^{-9} ng/kg body weight/day. For infants up to 6 months of age (the group with the greatest predicted exposure based on body weight), the total intake was estimated at 3.6×10^{-8} ng/kg-body weight/day. A study by Paraiba et al. (2006) calculated a BCF for 3,3'-dichlorobenzidine in fruits. Average daily intake values were not measured in this study, but the BCF calculated makes calculations of average daily intake values possible.

The study suggests that consumer exposure to 3,3'-dichlorobenzidine through fruit consumption is possible (Paraiba et al. 2006).

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

In addition to individuals who are occupationally exposed to 3,3'-dichlorobenzidine (see Section 5.6) there are other groups whose levels are above those of the general population. These groups include individuals living in proximity to sites where 3,3'-dichlorobenzidine was produced or sites where 3,3'-dichlorobenzidine was disposed of, and individuals living near one of the NPL hazardous waste sites where 3,3'-dichlorobenzidine has been detected in some environmental media (ATSDR 2019). Harden et al. (2005) found 3,3'-dichlorobenzidine and its degradation product, benzidine, present in sediment samples up to 6 km from its primary source indicating the chemical can be transported long distances. This suggests that the chemical and its products may be in local soils and sediments at elevated levels.

CHAPTER 6. ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 3,3'-dichlorobenzidine is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the adverse health effects (and techniques for developing methods to determine such health effects) of 3,3'-dichlorobenzidine.

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

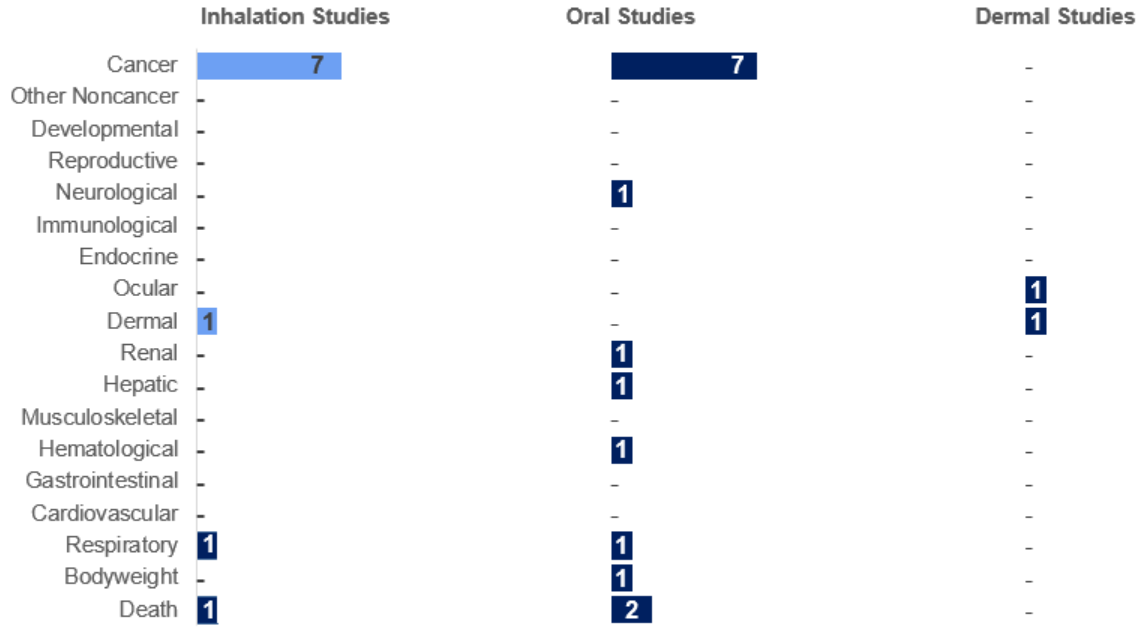
6.1 INFORMATION ON HEALTH EFFECTS

Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to 3,3'-dichlorobenzidine that are discussed in Chapter 2 are summarized in Figure 6-1. The purpose of this figure is to illustrate the information concerning the health effects of 3,3'-dichlorobenzidine. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

Figure 6-1 illustrates that a majority of the toxicity data available for 3,3'-dichlorobenzidine comes from oral studies on laboratory animals. Cancer is the most commonly studied endpoint. Very few studies were found on humans, and the majority of these studies examined cancer outcomes among occupationally exposed groups. Dermal and inhalation studies focused on a very small number of endpoints, as oral studies examined seven different endpoints, followed by inhalation and dermal, examining five and three endpoints, respectively.

Figure 6-1. Summary of Existing Health Effects Studies on 3,3'-Dichlorobenzidine By Route and Endpoint*

Potential cancer and hematological effects were the most studied endpoints
The majority of the studies examined oral exposure in **animals** (versus **humans**)



*Includes studies discussed in Chapter 2; the number of studies include those finding no effect; some studies examined multiple endpoints

6.2 IDENTIFICATION OF DATA NEEDS

Missing information in Figure 6-1 should not be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Acute-Duration MRLs. One study in humans showed that application of 3,3'-dichlorobenzidine base causes skin irritation (Gerarde and Gerarde 1974). The limited information in humans is insufficient to conclusively identify target organs, other than the skin, following exposure by any route. Acute-duration exposure can cause eye damage (erythema, pus, cornea1 opacity) in rabbits following conjunctival application. However, the relevance of these findings for the general population is unknown, since conjunctival application is not a likely route of exposure, and inhalation exposure is unlikely. 3,3'-Dichlorobenzidine can be lethal following oral and dermal exposure at very high doses. In most animal studies, comprehensive gross and histopathological evaluations have not been conducted, and clinical signs have not been monitored. Such studies may provide insight into systemic toxicity and potential health threats associated with acute-duration exposure. With the exception of effects caused by direct contact of 3,3'-dichlorobenzidine with the skin or the eyes, the limited pharmacokinetic data do not suggest route-specific target organs. The available data were inadequate for derivation of either inhalation or oral acute-duration MRLs.

Intermediate-Duration MRLs. No intermediate-duration studies in humans were located. Intermediate-duration oral studies have been performed in rats but no adverse systemic effects were reported. However, only one dose level was examined in all studies reviewed (Griswold et al. 1968; Ito et al. 1983; Osanai 1976; Pliss 1959, 1963). Organs and/or tissues from the reproductive, neurological, and immunological systems have not been examined in the available intermediate-duration studies; such information would be useful. No intermediate-duration inhalation or dermal studies were found. Animal studies evaluating toxicological parameters at several dose levels would provide dose-response data that could help better assess potential adverse effects in humans following intermediate-duration exposure. No oral intermediate-duration MRL was derived, because the available studies did not identify relevant noncancer effects.

Chronic-Duration MRLs. Studies examining noncancer endpoints in humans following chronic exposure to 3,3'-dichlorobenzidine are limited and insufficient for derivation of a chronic MRL. Available chronic-duration oral studies provide information regarding systemic and carcinogenic effects in rats and dogs (Stula et al. 1975, 1978). These studies employed one dose level and the toxicological parameters measured were limited. Serious effects were seen at the lowest dose tested. The inadequacies of these studies precluded derivation of a chronic oral MRL. No chronic-duration animal inhalation or dermal exposure studies were located. Well conducted chronic-duration inhalation, dermal, and oral studies involving low-dose exposure in animals might provide dose-response data on potential systemic effects that could be extrapolated to humans. The available data are insufficient to establish a relationship between the concentration of 3,3'-dichlorobenzidine and/or its metabolites in the body and the levels that are associated with adverse effects. Studies that provide data on the body burden of 3,3'-dichlorobenzidine associated with toxicity may prove useful.

Various studies have assessed the potential carcinogenicity of 3,3'-dichlorobenzidine in exposed workers (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Millerick-May et al. 2021; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996; Rosenman and Reilly 2004). However, many confounders have rendered the results inconclusive. A major difficulty in such studies is the simultaneous exposure to several potential or known carcinogens. The carcinogenicity of 3,3'-dichlorobenzidine has been well established in animals after oral administration of the compound (Osanai 1976; Pliss 1959, 1963; Stula et al. 1975, 1978), but no information is available regarding inhalation and dermal exposure. There is suggestive evidence that 3,3'-dichlorobenzidine may cause cancer in animals when applied dermally since tumors were found in rats injected with the compound subcutaneously (Pliss 1963). Of particular interest would be additional studies, using relevant routes of exposure, to confirm the findings that 3,3'-dichlorobenzidine causes cancer in offspring of rats injected with the chemical subcutaneously during pregnancy (Golub et al. 1975).

Health Effects. Few studies on human exposure to 3,3'-dichlorobenzidine were located, especially regarding noncancerous endpoints for all exposure routes. There is a need for studies examining noncancerous endpoints for 3,3'-dichlorobenzidine, particularly inhalation and dermal routes, which are more likely for workers. There is also need of human studies that identify the biological fate of 3,3'-dichlorobenzidine in the human body (see Absorption, Distribution, Metabolism, and Excretion below) to better distinguish if observed effects are due to the chemical. Additionally, no animal or human studies identified a specific target organ for 3,3'-dichlorobenzidine; studies examining this are needed.

Hepatic. Liver injury was seen in one of six dogs chronically exposed to 3,3'-dichlorobenzidine, noted by marked fatty change in the liver (Stula et al. 1978). All dogs exhibited modestly elevated ALT levels. Further intermediate- or chronic-duration oral animal studies testing a range of doses would help to determine the dose and time dependency of hepatotoxicity in animals.

Neurological. The nervous system has not been evaluated after exposure to 3,3'-dichlorobenzidine. A chronic-duration oral study in dogs reported convulsions in one of six dogs treated orally with 3,3'-dichlorobenzidine (Stula et al. 1978). Upon necropsy, the authors noticed slight neuronal degeneration in tissues (unspecified) of the nervous system from the convulsing dog. However, the effect was seen in only one of the six dogs and only one dose level was tested. The limited information available does not suggest that 3,3'-dichlorobenzidine is a neurotoxicant. However, any future long-term toxicity study on 3,3'-dichlorobenzidine in animals should include histological evaluation of representative elements of the nervous system. Furthermore, evaluation of neurological endpoints in offspring from animals exposed during gestation would provide information that may be relevant to children of pregnant women exposed to 3,3'-dichlorobenzidine in the workplace.

Developmental. Animal studies have shown that 3,3'-dichlorobenzidine and/or its metabolites may be transferred across the placenta and/or through maternal milk to the offspring and may affect the growth of the kidneys after parenteral exposure during pregnancy (Golub 1969; Shabad et al. 1972) or induce tumors in the offspring (Golub et al. 1975). Future animal studies examining various dose levels and relevant exposure routes during critical developmental periods may provide information on potential fetotoxicity, embryotoxicity, and teratogenic effects in humans. Also, cross-fostering studies may help determine the relative impacts of in utero transfer of the chemical and transfer through breast milk. Further animal data may provide dose-response information if studies are conducted to determine what dose of 3,3'-dichlorobenzidine, or its metabolites, reaches the fetus.

Cancer. Cancer studies of manufacturing workers suggest 3,3'-dichlorobenzidine may lead to the formation of bladder tumors and bladder cancer (Millerick-May et al. 2021; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996; Rosenman and Reilly 2004). Although these studies did not have information on cigarette smoking status, they were able to indirectly control for smoking by examining other smoking related cancers such as lung cancer, which were not statistically increased in the cohort (Rosenman and Reilly 2004). Moreover, they were not able to

distinguish between the effect of 3,3'-dichlorobenzidine or other chemicals that workers were exposed to (Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996). Studies on animals have also observed the development of tumors and carcinomas in various locations following oral exposure to 3,3'-dichlorobenzidine (Pliss 1959, 1963; Stula et al. 1975, 1978). At the same time, several studies did not report formations of tumors or carcinomas in animals, especially in the bladder (Griswold et al. 1968; Saffiotti et al. 1967). Additional studies of animals are needed to better establish whether exposure to 3,3'-dichlorobenzidine and the formation of cancerous tumors and carcinomas is an endpoint of concern among exposed workers.

Genotoxicity. Available studies in animals and in bacterial systems show that 3,3'-dichlorobenzidine did alter genetic material (Ashby and Mohammed 1988; Bratcher and Sikka 1982; Chen et al. 2003, 2014; Chung et al. 2000; Cihak and Vontorkova 1987; Garner et al. 1975; Hering et al. 2018; Iba 1987; Imaoka et al. 1997; Lazear et al. 1979; Makena and Chung 2007; Sasaki et al. 1999; Savard and Josephy 1986; Shiraishi 1986; Wang et al. 2005). Studies involving additional test systems may allow a better assessment of mutagenic potential.

Epidemiology and Human Dosimetry Studies. The potential for occupational exposure exists in the use of 3,3'-dichlorobenzidine in the synthesis of 3,3'-dichlorobenzidine-based pigments for printing ink applications and to a lesser extent in paints. Dermatitis is the only noncancerous adverse health effect that appears to be associated with 3,3'-dichlorobenzidine exposure attributed to a manufacturing process change that resulted in exposure to 3,3'-dichlorobenzidine-free base (Gerarde and Gerarde 1974). Studies of occupationally exposed individuals have been complicated by the fact that there is usually simultaneous exposure to other chemicals. Based on available data, the potential for nonindustrial exposure to the general population by air, soil, or water is expected to be negligible. Epidemiological studies of people who live in areas where 3,3'-dichlorobenzidine has been detected in groundwater, near industries releasing 3,3'-dichlorobenzidine, or near hazardous waste sites are not likely to provide information on whether 3,3'-dichlorobenzidine exposure produces effects in humans given the likelihood that the levels of exposure to these populations has been low. In the unlikely event that exposure of the general population (in the past or present) to 3,3'-dichlorobenzidine occurs, individuals should be monitored for dermal effects (as reported earlier by Gerarde and Gerarde 1974).

No studies were located that monitored human tissues for content of 3,3'-dichlorobenzidine or its metabolites. 3,3'-Dichlorobenzidine is excreted in urine. If 3,3'-dichlorobenzidine and metabolites can be

detected and correlated with exposure, it may be possible to correlate urinary levels of 3,3'-dichlorobenzidine or its metabolites, with systemic effects.

Biomarkers of Exposure and Effect. Methods for the determination of 3,3'-dichlorobenzidine in urine and serum have been reported (Birner et al. 1990; Bowman and Nony 1981; Knoell et al. 2012; Lee et al. 2003; Nony and Bowman 1980; Nony et al. 1980; Zwirner-Baier and Neumann 1994). Some of the methods have been shown to be suitable for the determination of the acetylated metabolites (Bowman and Nony 1981; Nony and Bowman 1980; Nony et al. 1980). The methods of Birner et al. (1990), Joppich-Kuhn et al. (1997), and Zwirner-Baier and Neumann (1994) permit the analysis of hemoglobin adducts of 3,3'-dichlorobenzidine and its monoacetyl metabolite. Monitoring of hemoglobin adducts combined with measuring urinary 3,3'-dichlorobenzidine concentrations showed that both tests together were effective tools of biological monitoring in humans (Knoell et al. 2012). Limits of detection for 3,3'-dichlorobenzidine in urine and serum were reported to be as low as 1–5 ppb (Bowman and Rushing 1981; Hofman and Schmidt 1993; Roberts and Rossano 1982), with detectable concentrations of the acetylated metabolites somewhat higher. Defining the levels of these biomarkers associated with exposures to 3,3'-dichlorobenzidine of toxicological concern can increase their utility.

There are no specific disease states in humans or animals that have been associated with exposure to 3,3'-dichlorobenzidine. Hemoglobin adducts have been isolated from the blood of 3,3'-dichlorobenzidine-treated animals (Birner et al. 1990; Joppich-Kuhn et al. 1997; Lee and Shin 2002) and exposed humans (Knoell et al. 2012). It is not known what relationship exists between adduct levels in the blood and 3,3'-dichlorobenzidine toxicity. Further research in animal models is needed to determine if these adducts could be correlated with effects of 3,3'-dichlorobenzidine exposure. Further studies to identify more sensitive toxic effects (noncancer) that are specific for 3,3'-dichlorobenzidine would be useful in monitoring effects in people living near hazardous waste sites containing 3,3'-dichlorobenzidine.

Absorption, Distribution, Metabolism, and Excretion. Available data are insufficient to allow accurate evaluation of absorption, metabolism, or persistence of 3,3'-dichlorobenzidine in human tissues. Additional studies to identify and quantify metabolites of 3,3'-dichlorobenzidine in humans and animals would be useful to establish the relevance of animal studies in predicting human health effects. Metabolic handling of 3,3'-dichlorobenzidine in humans needs to be better characterized to be able to utilize urinary levels of the compound or its metabolites to quantitate human exposure.

Comparative Toxicokinetics. Pharmacokinetics studies have not been performed under conditions analogous to those of the carcinogenicity studies. Therefore, it is not possible to determine systemic levels of the compound associated with the reported effects. Pharmacokinetic data developed under exposure conditions associated with biological effects would markedly increase the possibility of improved species extrapolation for evaluating the true potency of 3,3'-dichlorobenzidine in humans.

Children's Susceptibility. The information on health effects of 3,3'-dichlorobenzidine in humans is derived exclusively from studies of occupational exposure (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Millerick-May et al. 2021; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996; Rosenman and Reilly 2004). In one occupational study, it was reported that contact with the free base form of 3,3'-dichlorobenzidine caused dermatitis (Gerarde and Gerarde 1974); it is reasonable to assume that children will respond in a similar manner under similar exposure conditions, although such exposure scenarios for children seem unlikely. There is no information available to determine whether children and adults are equally susceptible to the toxic effects of 3,3'-dichlorobenzidine. No studies in animals have addressed this issue.

There is no information on whether the developmental process is altered in humans exposed to 3,3'-dichlorobenzidine. Studies in animals have been inadequate (Golub 1969; Golub et al. 1975; Shabad et al. 1972), and further well-conducted research would be helpful to clarify whether the developmental process can be affected in animals exposed to 3,3'-dichlorobenzidine by a relevant exposure route. This includes information on whether 3,3'-dichlorobenzidine (or its metabolites) can cross the placenta and/or be transferred to offspring via breast milk. There are no data to evaluate whether the pharmacokinetics of 3,3'-dichlorobenzidine in children is different from adults. There are no PBPK models for 3,3'-dichlorobenzidine. There is no information to evaluate whether metabolism of 3,3'-dichlorobenzidine in children is different than in adults.

Continued research into the development of sensitive and specific biomarkers of exposure and effect for 3,3'-dichlorobenzidine would be valuable. There are no biomarkers of exposure or effect for 3,3'-dichlorobenzidine that have been validated for children or adults exposed as children. There are no biomarkers in adults that identify previous childhood exposure. There are no data on interactions of 3,3'-dichlorobenzidine with other chemicals in children or adults. There are neither adult nor pediatric-specific methods to reduce peak absorption of 3,3'-dichlorobenzidine following exposure, to reduce body burdens, or to interfere with 3,3'-dichlorobenzidine's mechanism of action. However, it is reasonable to assume that if children have the potential to be exposed, avoidance measures should be used.

Physical and Chemical Properties. It has been demonstrated that 3,3'-dichlorobenzidine is strongly adsorbed by soils and sediments, and that it may not readily desorb. Adsorption cannot be accurately predicted a priori; such data are soil-system specific and must be determined experimentally for each system under study. Because there is some discrepancy regarding the volatility of the free base form of 3,3'-dichlorobenzidine (CPMA 1998; Gerarde and Gerarde 1974), research in this area is indicated.

Production, Import/Export, Use, Release, and Disposal. 3,3'-Dichlorobenzidine is no longer used to produce dyes in the United States (alternative dyes based on other chemicals are available) (NLM 2019). Its use is not expected to increase or return to past levels of use in the future due to its classification as a carcinogen. There is evidence that it can be brought into the home on the shoes and clothing of adults who work with 3,3'-dichlorobenzidine (ATSDR 1996) but the quantity that might be present is unknown. In the workplace, OSHA regulations require that 3,3'-dichlorobenzidine be handled in closed systems and that shipping containers be cleaned thoroughly (again, within a closed system) before disposal (DCMA 1989).

3,3'-Dichlorobenzidine is most likely to be found in sediments and soils near current or former industrial sites where the chemical was used. Since the chemical's use in U.S. manufacturing has decreased, releases to the environment are expected to be very low. Citations regarding disposal techniques for 3,3'-dichlorobenzidine are found in the Hazardous Substances Data Bank (NLM 2019). Small quantities can be destroyed by chemical reaction, for example, with sodium hypochlorite solution, which converts 3,3'-dichlorobenzidine to a quinone-type compound. Incineration at high temperatures can be used to destroy work garments and miscellaneous solid wastes exposed to the compound. Presumably, only small amounts would need to be disposed of since the compound is mainly consumed during the manufacture of pigments.

According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit chemical release and off-site transfer information to the EPA.

Environmental Fate. 3,3'-Dichlorobenzidine does not appear to biodegrade easily, but the few studies in this area did not state the type(s) or concentrations of microorganisms used in each study. More systematic studies with other organisms may prove useful. 3,3'-Dichlorobenzidine has been observed up to 6 km from its primary source, suggesting that the chemical travels when bound to sediments. Further

research on the chemical's ability to transport would provide needed data on exposure levels to the general population, especially from contact with contaminated soils.

A study by Nyman et al. 1997 provides evidence that in the span of a year, up to 80% of 3,3'-dichlorobenzidine can degrade to benzidine in anaerobic mixtures of sediment/water. Further research to identify the pathways and products of decomposition of 3,3'-dichlorobenzidine in various soils is needed.

Bioavailability from Environmental Media. The Canadian Government's Priority Substances List Assessment Report for 3,3'-dichlorobenzidine (Government of Canada 1993) reported that no data on the levels of 3,3'-dichlorobenzidine in drinking water or foodstuffs were identified within either Canada or the United States. 3,3'-Dichlorobenzidine has been found to bind strongly to soil constituents (Berry and Boyd 1985; Chung and Boyd 1987; Harden et al. 2005). Law (1995) concluded that it would also bind strongly to sedimentary material in the marine aquatic environment and may therefore have limited bioavailability. A study by Paraiba et al. (2006) suggested that 3,3'-dichlorobenzidine has the potential to bioconcentrate in orange orchards and its fruit, when the soil used is cultivated in sludge from industrial wastewater effluents. Further research into the chemical's bioavailability in fruits and other vegetation would provide evidence of possible exposure levels for the general public.

Food Chain Bioaccumulation. 3,3'-Dichlorobenzidine is bioconcentrated by aquatic organisms under experimental conditions. Whole-fish BCFs of around 500, with equilibration occurring in 96–168 hours, have been published (Appleton and Sikka 1980). In view of the n-octanol water partition coefficient for 3,3'-dichlorobenzidine, limited bioaccumulation could be expected (Law 1995) because the retention time of the chemical in exposed fish is short (Appleton and Sikka 1980). The ability of aquatic organisms to concentrate the compound could present a human health hazard if contaminated fish were eaten. However, 3,3'-dichlorobenzidine was not found in fish taken from waters in the vicinity of dye or textile manufacturing plants on the Buffalo and Delaware rivers in the United States (Diachenko 1979). It was concluded that monitoring for 3,3'-dichlorobenzidine in marine waters of the United Kingdom is unwarranted at present (Law 1995).

Exposure Levels in Environmental Media. There were no quantitative data on current atmospheric levels of 3,3'-dichlorobenzidine emissions or on the chemical's potential to act as a surface contaminant of soil environments. It is difficult to determine 3,3'-dichlorobenzidine levels in the aquatic environment because the concentrations tend to be at or below analytical detection limits. In general, it may only be possible to ascertain fully the environmental fate of 3,3'-dichlorobenzidine as analytical advances permit

the routine determination of very low concentrations. Moreover, determination of the nature and environmental fate of breakdown products of 3,3'-dichlorobenzidine would be useful. Reliable monitoring data for the levels of 3,3'-dichlorobenzidine in contaminated media at hazardous waste sites are needed so that the information obtained on levels of 3,3'-dichlorobenzidine in the environment can be used in combination with the known body burdens of 3,3'-dichlorobenzidine to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. It has been speculated that the 1974 OSHA regulations have reduced workplace air levels of 3,3'-dichlorobenzidine (CPMA 1998). However, it would be important to conduct exposure studies to monitor air levels in the workplace or conduct biomonitoring in workers to confirm this premise. There is continued need for more information on the extent of air, water, and soil contamination by industrial plant emissions or waste sites containing 3,3'-dichlorobenzidine. There is little information on exposure of children to 3,3'-dichlorobenzidine (or products derived from the compound). The compound has a very limited distribution and has only been detected in consumer goods sold abroad (other than in insoluble pigmented forms). Exposure may also occur from dietary sources, including aquatic organisms and fruit, but extent of the exposure is unknown. This information can be useful for assessing the need to conduct health studies on these populations.

Exposures of Children. There is no available information on exposure of children to 3,3'-dichlorobenzidine (or products derived from the compound). The compound has been found in trace amounts in some cosmetics, skin care, and personal hygiene products available for consumer use abroad (Hailong et al. 2014). Quantifying child exposure to the chemical by dermal use of these products, or accidental ingestion, would be useful in determining if exposure is of concern. Inadvertent take-home exposure by occupationally exposed parents could also be explored. A public health assessment (ATSDR 1996) found measurable levels of 3,3'-dichlorobenzidine (10.5 ppm in vacuum cleaner bags and 0.74 ppm in clothes dryer lint) in the homes of workers who were employed in manufacturing or processing the compound.

6.3 ONGOING STUDIES

No ongoing studies were identified for 3,3'-dichlorobenzidine during the literature search or in the NIH Research Portfolio Online Reporting Tools (RePORTER 2021).

CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding 3,3'-dichlorobenzidine in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency.

ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the MRLs for 3,3'-dichlorobenzidine.

Table 7-1. Regulations and Guidelines Applicable to 3,3'-Dichlorobenzidine

Agency	Description	Information	Reference
	Air		
EPA	RfC	Not evaluated	IRIS 2006
WHO	Air quality guidelines	No data	WHO 2010
	Water & Food		
EPA	Drinking water standards and health advisories	Not listed	EPA 2018b
	National primary drinking water regulations	Not listed	EPA 2009
	RfD	Reviewed but inadequate data	IRIS 2006
WHO	Drinking water quality guidelines	No data	WHO 2017
FDA	Substances Added to Food[a]	Not listed	FDA 2021
	Cancer		
HHS	Carcinogenicity classification	Reasonably anticipated to be a human carcinogen	NTP 2021
EPA	Carcinogenicity classification	Group B2[b]	IRIS 2006
	Oral slope factor	$4.5X10^{-1}$ mg/kg-day	
IARC	Carcinogenicity classification	Group 2B[c]	IARC 1987
	Occupational		
OSHA	PEL (8-hour TWA) for general industry, shipyards and construction	No PEL established; Potential occupational carcinogen; Exposure to be controlled through the required use of engineering controls, work practices, and personal protective equipment	OSHA 2021a, OSHA 2021b

Table 7-1. Regulations and Guidelines Applicable to 3,3'-Dichlorobenzidine

Agency	Description	Information	Reference
NIOSH	REL (up to 10-hour TWA)	No REL established; Potential occupational carcinogen; Exposures to carcinogens be limited to the lowest feasible concentration	NIOSH 2019
	Emergency Criteria		
EPA	AEGLs-air	No data	EPA 2018a
DOE	PACs-air		DOE 2018a
	PAC-1[d]	2.1 ppm	
	PAC-2[d]	23 ppm	
	PAC-3[d]	140 ppm	

[a]The Substances Added to Food inventory replaces EAFUS and contains the following types of ingredients: food and color additives listed in FDA regulations, flavoring substances evaluated by FEMA or JECFA, GRAS substances listed in FDA regulations, substances approved for specific uses in food prior to September 6, 1958, substances that are listed in FDA regulations as prohibited from use in food, delisted color additives, and some substances "no longer FEMA GRAS".
[b]Group B2: Probable human carcinogen.
[c]Group 2B: Possibly carcinogenic to humans.
[d]Definitions of PAC terminology are available from U.S. Department of Energy (DOE 2018b).

AEGL = acute exposure guideline levels; DOE = Department of Energy; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; FEMA = Flavor and Extract Manufacturers Association of the United States; GRAS = Generally Recognized As Safe; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; JECFA = Joint FAO/WHO Expert Committee on Food Additives; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = Protective Action Criteria; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TWA = time-weighted average; WHO = World Health Organization

CHAPTER 8. REFERENCES

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APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

APPENDIX A

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Office of Innovation and Analytics, Toxicology Section, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Office of Innovation and Analytics, Toxicology Section, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S102-1, Atlanta, Georgia 30329-4027.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 3,3'-Dichlorobenzidine
CAS Numbers: 91-94-1
Date: June 2022
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL due to the lack of quantitative exposure-response data for humans or animals.

Rationale for Not Deriving an MRL: Only a single review study describes acute inhalation exposure in animals and humans (Gerarde and Gerarde 1974). The animal study reported in the review is severely limited due to lack of detailed reporting of the results. The reported human results in Gerarde and Gerarde (1974) are limited due to non-reporting of exposure concentration, concurrent exposure to other carcinogens, and failure to use control groups. Additionally, the primary endpoint examined was cancer, which is not used to derive an MRL.

Agency Contacts (Chemical Managers): Custodio Muianga

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 3,3'-Dichlorobenzidine
CAS Numbers: 91-94-1
Date: June 2022
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL due to the lack of studies examining this exposure duration in humans and animals.

Rationale for Not Deriving an MRL: No human or animal studies were located that examined intermediate duration inhalation exposure to 3,3'-dichlorobenzidine. Subsequently, no MRL has been derived.

Agency Contacts (Chemical Managers): Custodio Muianga

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 3,3'-Dichlorobenzidine
CAS Numbers: 91-94-1
Date: June 2022
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL due to the lack of animal studies examining chronic duration inhalation exposure to 3,3'-dichlorobenzidine, and the lack of quantitative human data.

Rationale for Not Deriving an MRL: The available chronic-inhalation studies in humans do not provide quantitative exposure information and primarily evaluate cancer as an endpoint, which cannot be used to derive an MRL (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Myslak et al. 1991). No animal studies were located that examined chronic inhalation to 3,3'-dichlorobenzidine.

Agency Contacts (Chemical Managers): Custodio Muianga

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 3,3'-Dichlorobenzidine
CAS Numbers: 91-94-1
Date: June 2022
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for 3,3'-dichlorobenzidine due to the lack of quantitative exposure-response data in humans or animals.

Rationale for Not Deriving an MRL: No human studies examined acute-duration oral exposure to 3,3'-dichlorobenzidine; therefore, insufficient data exist to derive an MRL. The available animal studies evaluate genotoxicity (Ashby and Mohammed 1988; Cihak and Vontorkova 1987; Ghosal and Iba 1990). Gerarde and Gerarde (1974) and Gaines and Nelson (as cited in EPA 1980a) only reported LD_{50} values in animals following acute-duration oral exposure. There was an overall lack of suitable data to derive an MRL.

Agency Contacts (Chemical Managers): Custodio Muianga

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 3,3'-Dichlorobenzidine
CAS Numbers: 91-94-1
Date: June 2022
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for 3,3'-dichlorobenzidine due to the lack of studies on humans and the lack of exposure-response data in animals.

Rationale for Not Deriving an MRL: No human studies were located examining oral intermediate-duration exposure to 3,3'-dichlorobenzidine. Oral intermediate-duration studies on animals primarily evaluated the cancer endpoint, with no other effects reported (Griswold et al. 1968; Ito et al. 1983; Osanai 1976; Pliss 1959, 1963). No MRL is derived due to the lack of suitable data.

Agency Contacts (Chemical Managers): Custodio Muianga

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** 3,3'-Dichlorobenzidine
CAS Numbers: 91-94-1
Date: June 2022
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for 3,3'-dichlorobenzidine due to the lack of studies in humans and animals examining noncancer endpoints.

Rationale for Not Deriving an MRL: No human studies were located that examined noncancer endpoints in humans following chronic-duration oral exposure to 3,3'-dichlorobenzidine. Chronic-duration oral studies in animals examine carcinogenic effects in rats and dogs (Stula et al. 1975, 1978). Stula et al. (1978) reported convulsions and neuronal degeneration in one of six dogs; however, this endpoint is a serious LOAEL, and therefore, no MRL can be derived using this endpoint.

Agency Contacts (Chemical Managers): Custodio Muianga

APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR 3,3'-DICHLOROBENZIDINE

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to 3,3'-dichlorobenzidine.

B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen was conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for 3,3'-dichlorobenzidine. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of 3,3'-dichlorobenzidine have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of 3,3'-dichlorobenzidine are presented in Table B-1.

Table B-1. Inclusion Criteria for the Literature Search and Screen

Health Effects
Species
Human
Laboratory mammals
Route of exposure
Inhalation
Oral
Dermal (or ocular)
Parenteral (these studies will be considered supporting data)
Health outcome
Death
Systemic effects
Body weight effects
Respiratory effects
Cardiovascular effects
Gastrointestinal effects
Hematological effects
Musculoskeletal effects
Hepatic effects
Renal effects
Dermal effects
Ocular effects
Endocrine effects
Immunological effects
Neurological effects
Reproductive effects

Table B-1. Inclusion Criteria for the Literature Search and Screen

Developmental effects
Other noncancer effects
Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models
Biomarkers
Biomarkers of exposure
Biomarkers of effect
Interactions with other chemicals
Potential for human exposure
Releases to the environment
Air
Water
Soil
Environmental fate
Transport and partitioning
Transformation and degradation
Environmental monitoring
Air
Water
Sediment and soil
Other media
Biomonitoring
General populations
Occupation populations

B.1.1 Literature Search

The current literature search was intended to update the draft toxicological profile for 3,3'-dichlorobenzidine released for public comment in 2021; thus, the literature search was restricted to studies published between January 2018 and November 2021. The following main databases were searched in November 2021:

- PubMed
- National Technical Reports Library (NTRL)
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, Medical Subject Headings (MeSH) headings, and keywords for 3,3'-dichlorobenzidine. The query strings used for the literature search are presented in Table B-2.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures and Results (NIH RePORTER) databases using the queries presented in Table B-3. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance Priority List (SPL) resource page, and other items as needed. Regulations applicable to 3,3'-dichlorobenzidine were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

Table B-2. Database Query Strings

Database search date	Query string
PubMed	
11/2021	("3,3'-Dichlorobenzidine"[mh] OR "(1,1'-Biphenyl)-4,4'-diamine, 3,3'-dichloro-"[tw] OR "3,3'-Dichloro-(1,1'-biphenyl)-4,4'-diamine"[tw] OR "3,3'-Dichloro-4,4'-biphenyldiamine"[tw] OR "3,3'-Dichloro-4,4'-diamino(1,1-biphenyl)"[tw] OR "3,3'-Dichloro-4,4'-diaminobiphenyl"[tw] OR "3,3'-Dichloro-4,4'-diaminodiphenyl"[tw] OR "3,3'-Dichloro-[1,1'-biphenyl]-4,4'-diamine"[tw] OR "3,3'-Dichloro-p,p'-bianiline"[tw] OR "3,3'-Dichloro[1,1'-biphenyl]-4,4'-diamine"[tw] OR "3,3'-Dichlorobenzidine"[tw] OR "3,3'-Dichlorobiphenyl-4,4'-diamine"[tw] OR "4-(4-amino-3-chlorophenyl)-2-chloroaniline"[tw] OR "4'-Amino-3,3'-dichloro(1,1'-biphenyl)-4-ylamine"[tw] OR "4,4'-Diamino-3,3'-dichlorobiphenyl"[tw] OR "4,4'-Diamino-3,3'-dichlorodiphenyl"[tw] OR "4,4′-Bis(2-chloroaniline)"[tw] OR "4,4′-Diamino-3,3′-dichlorobiphenyl"[tw] OR "4,4′-Diamino-3,3′-dichlorodiphenyl"[tw] OR "4′-Amino-3,3′-dichloro[1,1′-biphenyl]-4-ylamine"[tw] OR "[1,1'-biphenyl]-4,4'-diamine, 3,3'-dichloro-"[tw] OR "Benzidine, 3,3'-dichloro-"[tw] OR "Curithane C 126"[tw] OR "Curithane C126"[tw] OR "Dichlodine H"[tw] OR "Dichlorobenzidine base"[tw] OR "o,o'-Dichlorobenzidine"[tw] OR "ortho,ortho'-Dichlorobenzidine"[tw] OR "Sulfuric acid--3,3'-dichloro[1,1'-biphenyl]-4,4'-diamine (1/1)"[tw] OR "Sulfuric acid--3,3'-dichloro[1,1'-biphenyl]-4,4'-diamine (2/1)"[tw] OR "1,1'-biphenyl-4,4'-diamine, 3,3'-dichloro-"[tw] OR "DICHLOROBENZIDINE"[tw] OR "AC1L3YN5"[tw] OR "AKOS030538069"[tw] OR "CTK5C1212"[tw] OR "LS-32418"[tw] OR "SCHEMBL8641898"[tw]) AND (2018/01/01:3000[dp] OR 2018/12/01:3000[mhda] OR 2018/12/01:3000[crdat] OR 2018/12/01:3000[edat])
NTRL	
11/2021	dichlorobenzidine "3,3'-Dichloro-(1,1'-biphenyl)-4,4'-diamine" "3,3'-Dichloro-4,4'-biphenyldiamine" "3,3'-Dichloro-4,4'-diamino(1,1-biphenyl)" "3,3'-Dichloro-4,4'-diaminobiphenyl" "3,3'-Dichloro-4,4'-diaminodiphenyl" "3,3'-Dichloro-[1,1'-biphenyl]-4,4'-diamine" "3,3'-Dichloro-p,p'-bianiline" "3,3'-Dichloro[1,1'-biphenyl]-4,4'-diamine" "3,3'-Dichlorobiphenyl-4,4'-diamine" "4'-Amino-3,3'-dichloro(1,1'-biphenyl)-4-ylamine" "4,4'-Diamino-3,3'-dichlorobiphenyl" "4,4'-Diamino-3,3'-dichlorodiphenyl" "4,4′-Bis(2-chloroaniline)"

Table B-2. Database Query Strings

Database search date	Query string
	"4,4′-Diamino-3,3′-dichlorobiphenyl" "4,4′-Diamino-3,3′-dichlorodiphenyl" "4-(4-amino-3-chlorophenyl)-2-chloroaniline" "4′-Amino-3,3′-dichloro[1,1′-biphenyl]-4-ylamine"
Toxcenter	
11/2021	FILE 'TOXCENTER' ENTERED AT 14:21:35 ON 22 NOV 2021 CHARGED TO COST=EH038.13.04.LB.04 L1 895 SEA FILE=TOXCENTER 91-94-1 OR 612-83-9 OR 64969-34-2 OR 64414-68-2 OR 74332-73-3 L2 872 SEA FILE=TOXCENTER L1 NOT TSCATS/FS L4 843 SEA FILE=TOXCENTER L2 NOT PATENT/DT L5 28 SEA FILE=TOXCENTER L4 AND PY>2017 L6 27 DUP REM L5 (1 DUPLICATE REMOVED) ANSWERS '1-27' FROM FILE TOXCENTER D SCAN L6

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS via ChemView	
11/2021	Compounds searched: 91-94-1, 612-83-9, 64969-34-2, 64414-68-2, 74332-73-3
NTP	
11/2021	91-94-1 612-83-9 64969-34-2 64414-68-2 74332-73-3 Dichlorobenzidine "3,3'-Dichloro-(1,1'-biphenyl)-4,4'-diamine" "3,3'-Dichloro-4,4'-biphenyldiamine" "3,3'-Dichloro-4,4'-diamino(1,1-biphenyl)" "3,3'-Dichloro-4,4'-diaminobiphenyl" "3,3'-Dichloro-4,4'-diaminodiphenyl" "3,3'-Dichloro-[1,1'-biphenyl]-4,4'-diamine" "3,3'-Dichloro-p,p'-bianiline" "3,3'-Dichloro[1,1'-biphenyl]-4,4'-diamine" "3,3'-Dichlorobiphenyl-4,4'-diamine" "4'-Amino-3,3'-dichloro(1,1'-biphenyl)-4-ylamine" "4,4'-Diamino-3,3'-dichlorobiphenyl" "4,4'-Diamino-3,3'-dichlorodiphenyl" "4,4′-Bis(2-chloroaniline)" "4,4′-Diamino-3,3′-dichlorobiphenyl" "4,4′-Diamino-3,3′-dichlorodiphenyl" "4-(4-amino-3-chlorophenyl)-2-chloroaniline" "4′-Amino-3,3′-dichloro[1,1′-biphenyl]-4-ylamine"
Regulations.gov	
11/2021	91-94-1 612-83-9 64969-34-2 64414-68-2 74332-73-3 dichlorobenzidine "3,3'-Dichloro-(1,1'-biphenyl)-4,4'-diamine"

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
	"3,3'-Dichloro-4,4'-biphenyldiamine" "3,3'-Dichloro-4,4'-diamino(1,1-biphenyl)" "3,3'-Dichloro-4,4'-diaminobiphenyl" "3,3'-Dichloro-4,4'-diaminodiphenyl" "3,3'-Dichloro-[1,1'-biphenyl]-4,4'-diamine" "3,3'-Dichloro-p,p'-bianiline" "3,3'-Dichloro[1,1'-biphenyl]-4,4'-diamine" "3,3'-Dichlorobiphenyl-4,4'-diamine" "4'-Amino-3,3'-dichloro(1,1'-biphenyl)-4-ylamine" "4,4'-Diamino-3,3'-dichlorobiphenyl" "4,4'-Diamino-3,3'-dichlorodiphenyl" "4,4′-Bis(2-chloroaniline)" "4,4′-Diamino-3,3′-dichlorobiphenyl" "4,4′-Diamino-3,3′-dichlorodiphenyl" "4-(4-amino-3-chlorophenyl)-2-chloroaniline" "4′-Amino-3,3′-dichloro[1,1′-biphenyl]-4-ylamine"
NIH RePORTER	
12/2021	Fiscal Year: Active Projects Text Search: "(1,1'-Biphenyl)-4,4'-diamine, 3,3'-dichloro-" OR "3,3'-Dichloro-(1,1'-biphenyl)-4,4'-diamine" OR "3,3'-Dichloro-4,4'-biphenyldiamine" OR "3,3'-Dichloro-4,4'-diamino(1,1-biphenyl)" OR "3,3'-Dichloro-4,4'-diaminobiphenyl" OR "3,3'-Dichloro-4,4'-diaminodiphenyl" OR "3,3'-Dichloro-[1,1'-biphenyl]-4,4'-diamine" OR "3,3'-Dichloro-p,p'-bianiline" OR "3,3'-Dichloro[1,1'-biphenyl]-4,4'-diamine" OR "3,3'-Dichlorobenzidine" OR "3,3'-Dichlorobiphenyl-4,4'-diamine" OR "4-(4-amino-3-chlorophenyl)-2-chloroaniline" OR "4'-Amino-3,3'-dichloro(1,1'-biphenyl)-4-ylamine" OR "4,4'-Diamino-3,3'-dichlorobiphenyl" OR "4,4'-Diamino-3,3'-dichlorodiphenyl" OR "4,4′-Bis(2-chloroaniline)" OR "4,4′-Diamino-3,3′-dichlorobiphenyl" OR "4,4′-Diamino-3,3′-dichlorodiphenyl" OR "4′-Amino-3,3′-dichloro[1,1′-biphenyl]-4-ylamine" OR "[1,1'-biphenyl]-4,4'-diamine, 3,3'-dichloro-" OR "Benzidine, 3,3'-dichloro-" OR "Curithane C 126" OR "Curithane C126" OR "Dichlodine H" OR "Dichlorobenzidine base" OR "o,o'-Dichlorobenzidine" OR "ortho,ortho'-Dichlorobenzidine" OR "Sulfuric acid--3,3'-dichloro[1,1'-biphenyl]-4,4'-diamine (1/1)" OR "Sulfuric acid--3,3'-dichloro[1,1'-biphenyl]-4,4'-diamine (2/1)" OR "1,1'-biphenyl-4,4'-diamine, 3,3'-dichloro-" OR "DICHLOROBENZIDINE" (advanced) Limit to: Project Title, Project Terms, Project Abstracts
Other	Identified throughout the assessment process

The 2021 results were:

- Number of records identified from PubMed, NTRL, and TOXCENTER (after duplicate removal): 28
- Number of records identified from other strategies: 14
- Total number of records to undergo literature screening: 42

B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on 3,3'-dichloro-benzidine:

- Title and abstract screen
- Full text screen

Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

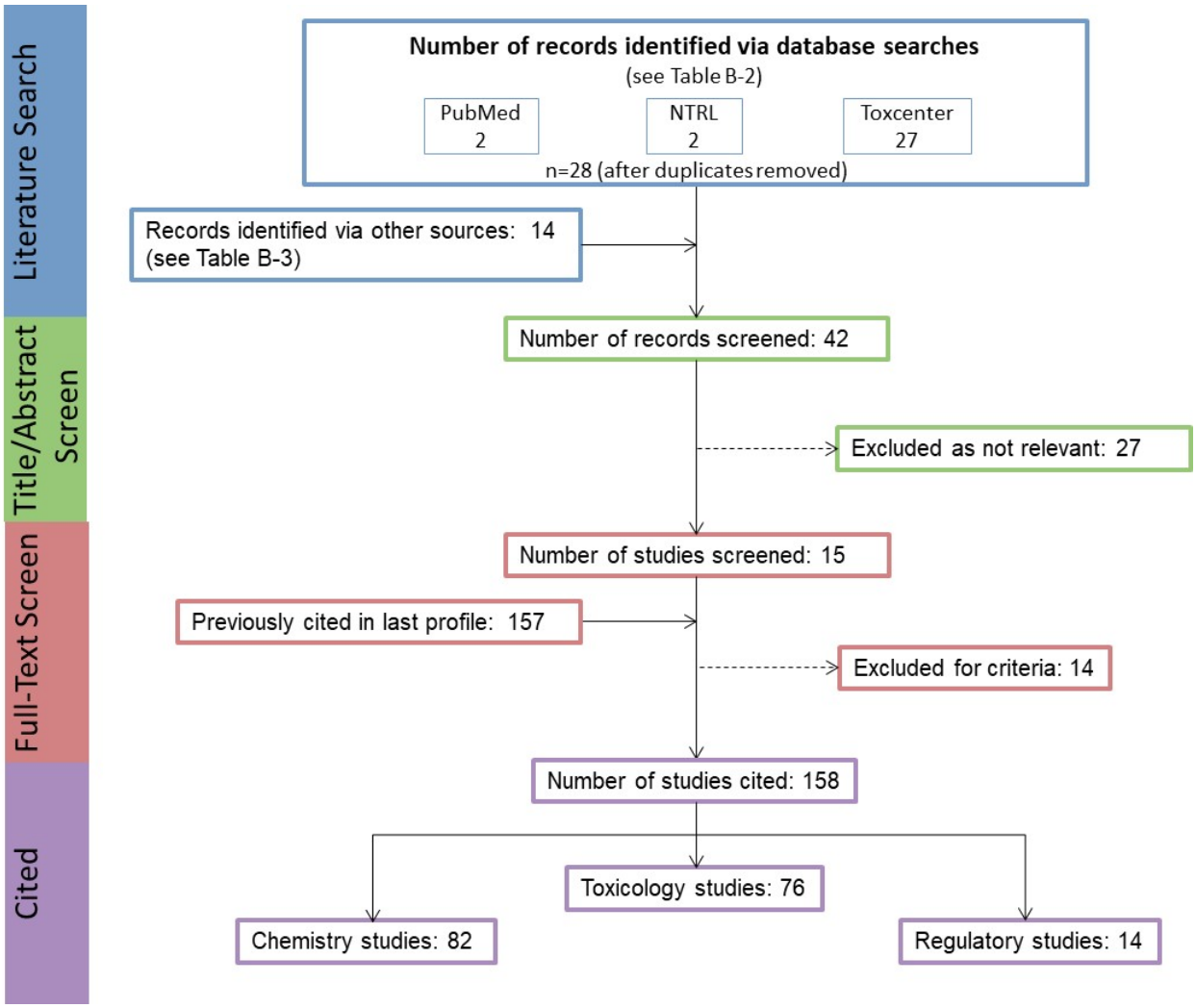
- Number of titles and abstracts screened: 42
- Number of studies considered relevant and moved to the next step: 15

Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 15
- Number of studies cited in the pre-public draft of the toxicological profile: 157
- Total number of studies cited in the profile: 158

A summary of the results of the literature search and screening is presented in Figure B-1.

Figure B-1. November 2021 Literature Search Results and Screen for 3,3'-Dichlorobenzidine



APPENDIX C. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page C-5)

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), food intake (FI), gross necropsy (GN), hematology (HE), histopathology (HP), immune function (IX), lethality (LE), neurological function (NX), organ function (OF), ophthalmology (OP), organ weight (OW), reproductive function (RX), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

FIGURE LEGEND

See Sample LSE Figure (page C-6)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(12) Exposure period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the chronic exposure period are illustrated.

(13) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

(14) Levels of exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(15) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(16) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

(17) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

APPENDIX C

Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral (1)

Figure key[a] (4)	Species (strain) No./group (4)	Exposure parameters (5)	Doses (mg/kg/day) (5)	Parameters monitored (6)	Endpoint (7)	NOAEL (mg/kg/day) (8)	Less serious LOAEL (mg/kg/day) (9)	Serious LOAEL (mg/kg/day) (9)	Effect
CHRONIC EXPOSURE (2)									
51 (3)	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4	CS, WI, BW, OW, HE, BC, HP	Bd wt	25.5	138.0		Decreased body weight gain in males (23–25%) and females (31–39%)
					Hemato	138.0			
					Hepatic		6.1[c]		Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure
Aida et al. 1992 (10)									
52	Rat (F344) 78 M	104 weeks (W)	0, 3.9, 20.6, 36.3	CS, BW, FI, BC, OW, HP	Hepatic	36.3			
					Renal	20.6	36.3		Increased incidence of renal tubular cell hyperplasia
					Endocr	36.3			
George et al. 2002									
59	Rat (Wistar) 58M, 58F	Lifetime (W)	M: 0, 90 F: 0, 190	BW, HP	Cancer		190 F		Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided
Tumasonis et al. 1985									

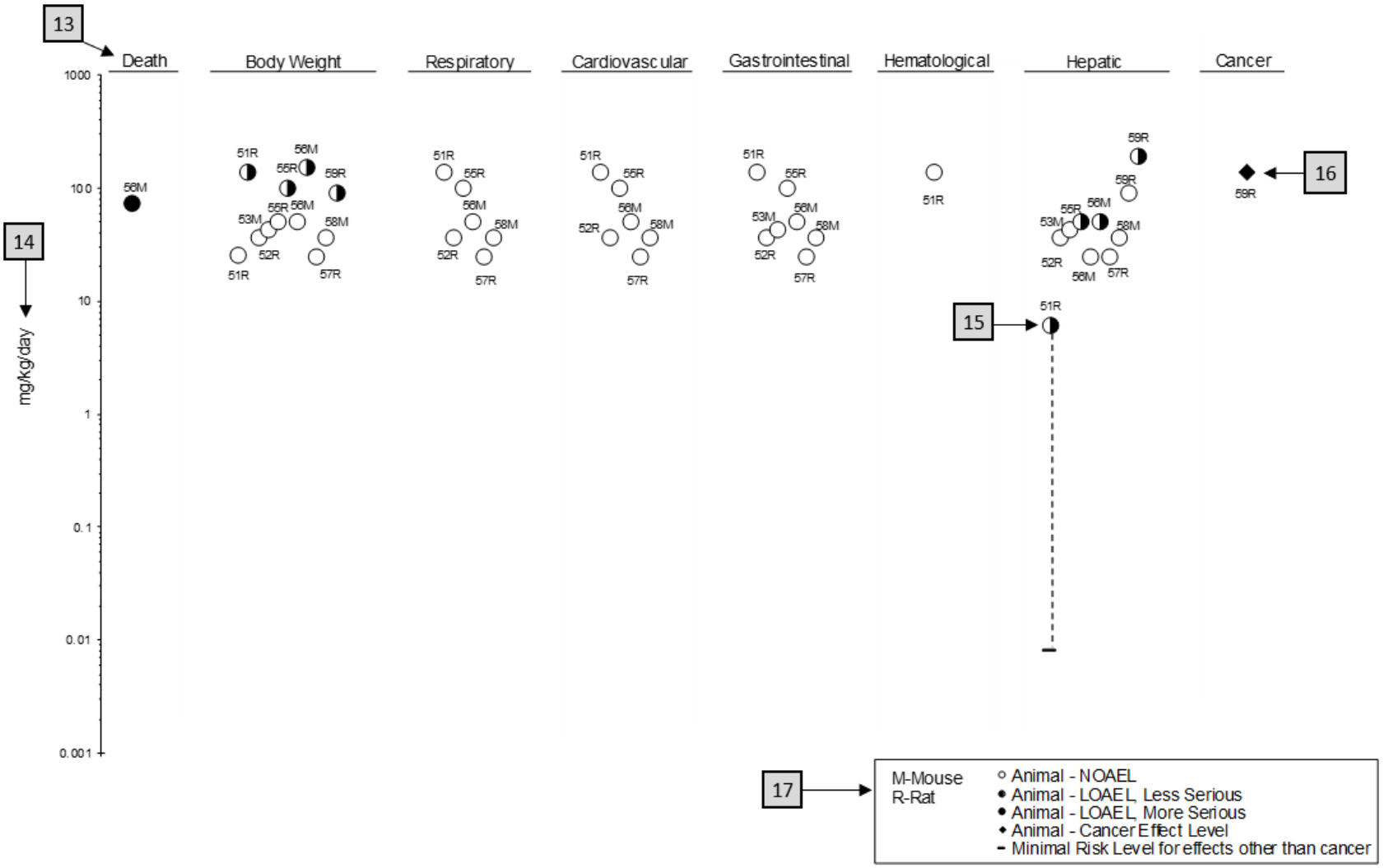
[a]The number corresponds to entries in Figure 2-x.

(11) [b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

APPENDIX C

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral



APPENDIX D. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Relevance to Public Health: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

Chapter 2: Health Effects: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting.

Pediatrics:
Section 3.2 Children and Other Populations that are Unusually Susceptible
Section 3.3 Biomarkers of Exposure and Effect

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

ATSDR develops educational and informational materials for health care providers categorized by hazardous substance, clinical condition, and/or by susceptible population. The following additional materials are available online:

Physician Briefs discuss health effects and approaches to patient management in a brief/factsheet style. *Physician Overviews* are narrated PowerPoint presentations with Continuing Education credit available (see https://www.atsdr.cdc.gov/emes/health_professionals/index.html).

Managing Hazardous Materials Incidents is a set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.html).

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

APPENDIX E. GLOSSARY

Absorption—The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD) or Benchmark Concentration (BMC)—is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a BMD_{10} would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

Cancer Effect Level (CEL)—The lowest dose of a chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

Clastogen—A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

Epidemiology—The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Excretion—The process by which metabolic waste products are removed from the body.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

Health Advisory—An estimate of acceptable drinking water levels for a chemical substance derived by EPA and based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunotoxicity—Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Metabolism—Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

Mortality—Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Although effects may be produced at this dose, they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) ≥1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio/Relative Risk—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxicokinetic—The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

Toxics Release Inventory (TRI)—The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX F. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AAPCC	American Association of Poison Control Centers
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ACMT	American College of Medical Toxicology
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Level
AIC	Akaike's information criterion
AIHA	American Industrial Hygiene Association
ALT	alanine aminotransferase
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BCF	bioconcentration factor
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
C	centigrade
CAA	Clean Air Act
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
cm	centimeter
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DWEL	drinking water exposure level
EAFUS	Everything Added to Food in the United States
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
ERPG	emergency response planning guidelines
F	Fahrenheit
F1	first-filial generation
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	Federal Register

FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GGT	γ-glutamyl transferase
GRAS	generally recognized as safe
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Level of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
MRL	Minimal Risk Level
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
Mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NCEH	National Center for Environmental Health
ND	not detected
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences

NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEHSU	Pediatric Environmental Health Specialty Unit
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
pg	picogram
PND	postnatal day
POD	point of departure
ppb	parts per billion
ppbv	parts per billion by volume
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure limit
REL-C	recommended exposure level-ceiling value
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification
SLOAEL	serious lowest-observed-adverse-effect level
SMR	standardized mortality ratio
sRBC	sheep red blood cell
STEL	short term exposure limit
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey

USNRC	U.S. Nuclear Regulatory Commission
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result