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:

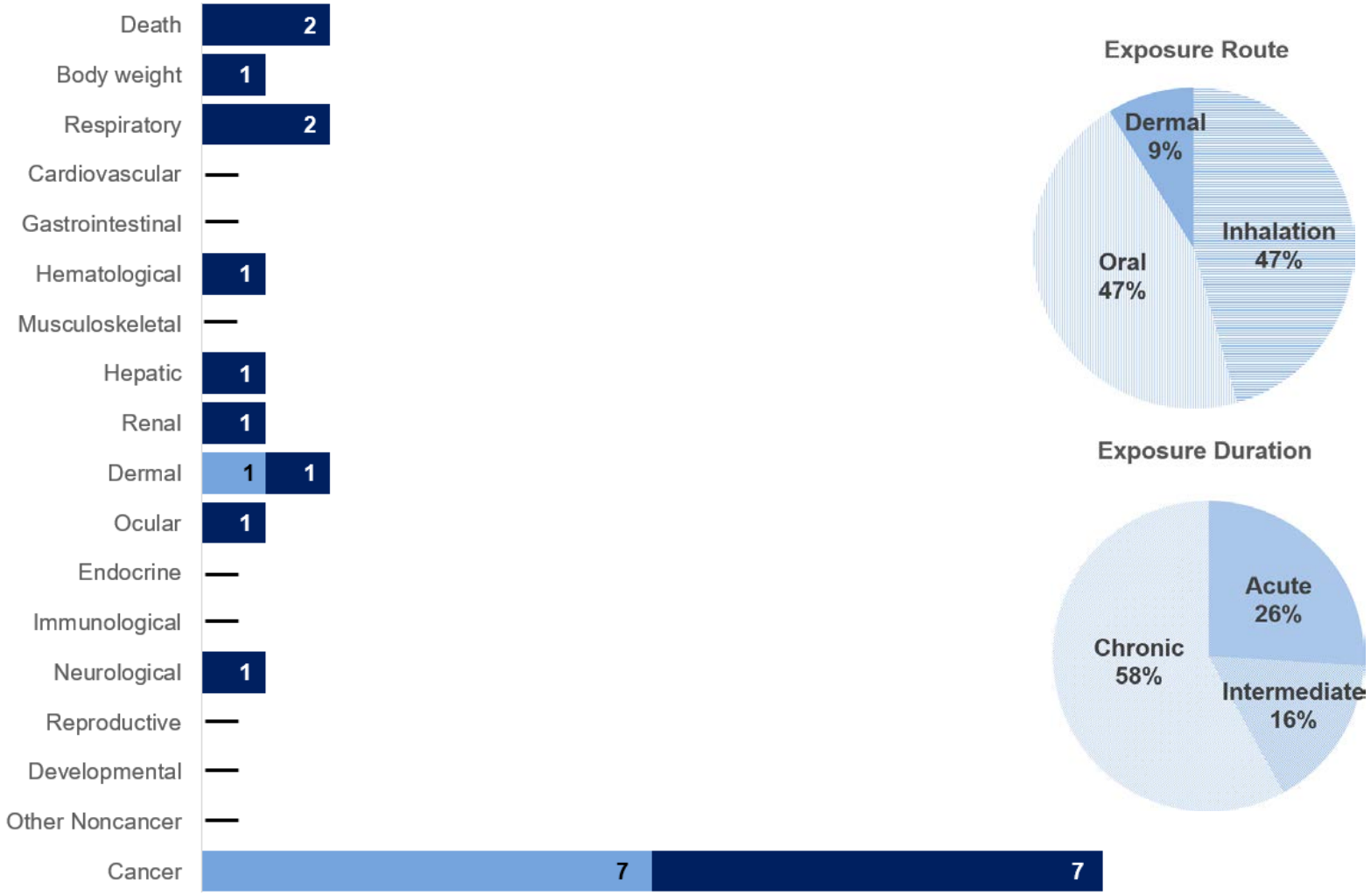
- **Cancer.** Human evidence for cancer is limited to epidemiological studies in occupationally exposed populations. Bladder tumors and increased risk for lymphohematopoietic cancer were identified. Most studies found no association between exposure and bladder cancer incidence. The epidemiological data 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)

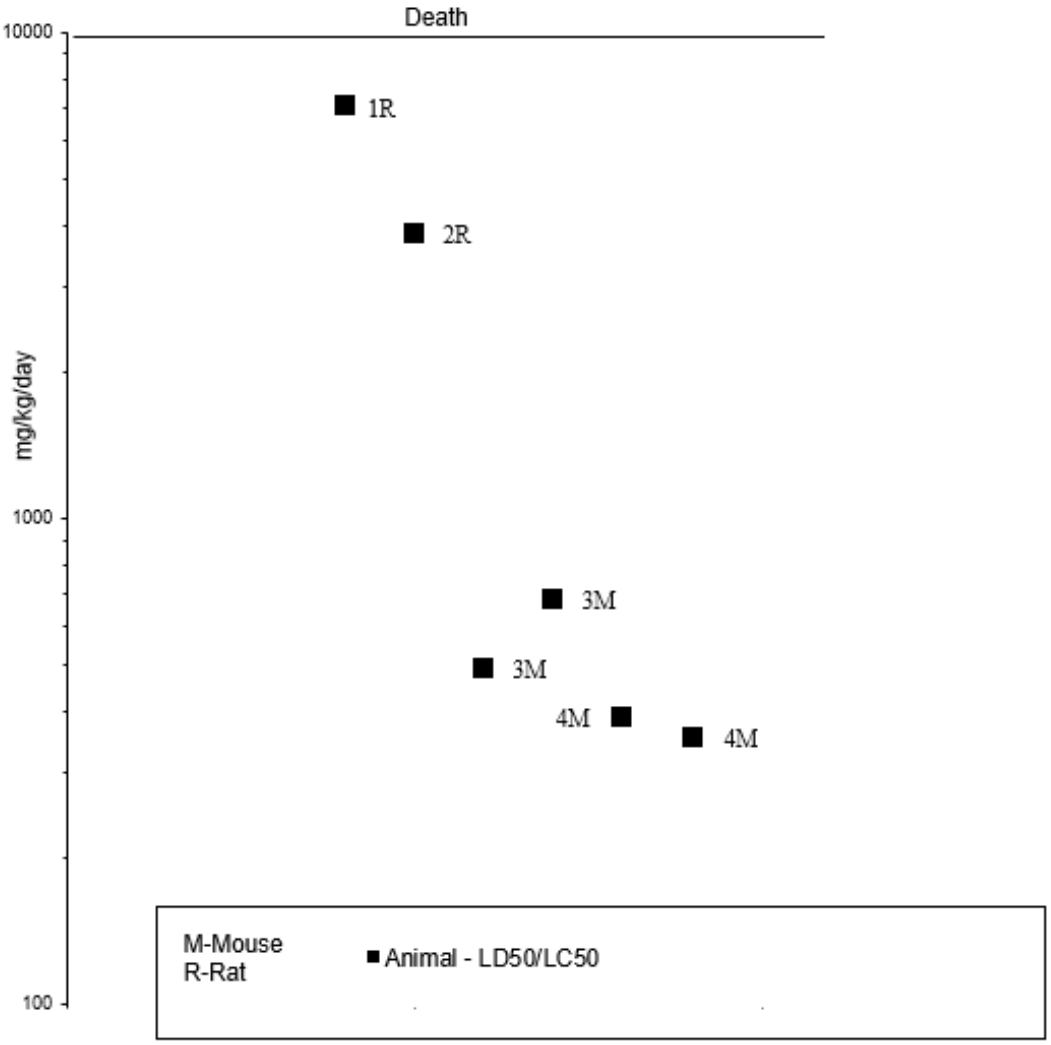


Figure 2-2. Levels of Significant Exposure to 3,3'-Dichlorobenzidine – Oral

Intermediate (15-364 days)

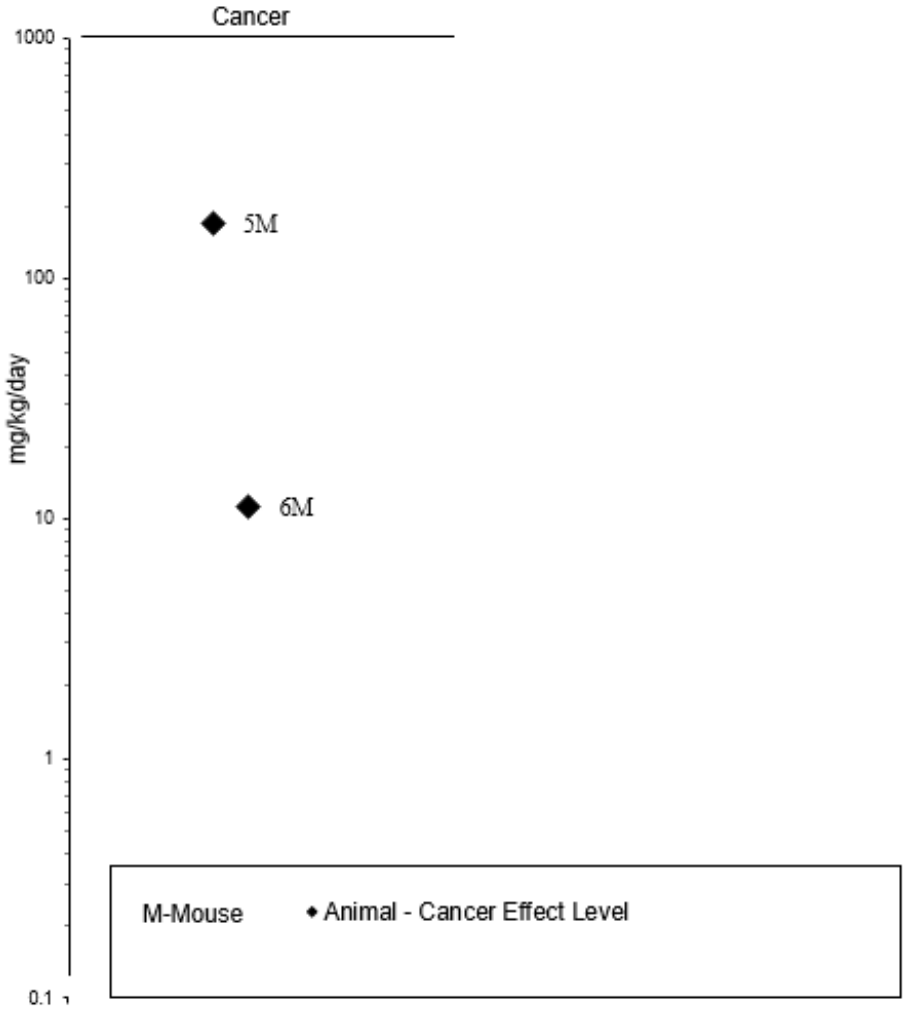
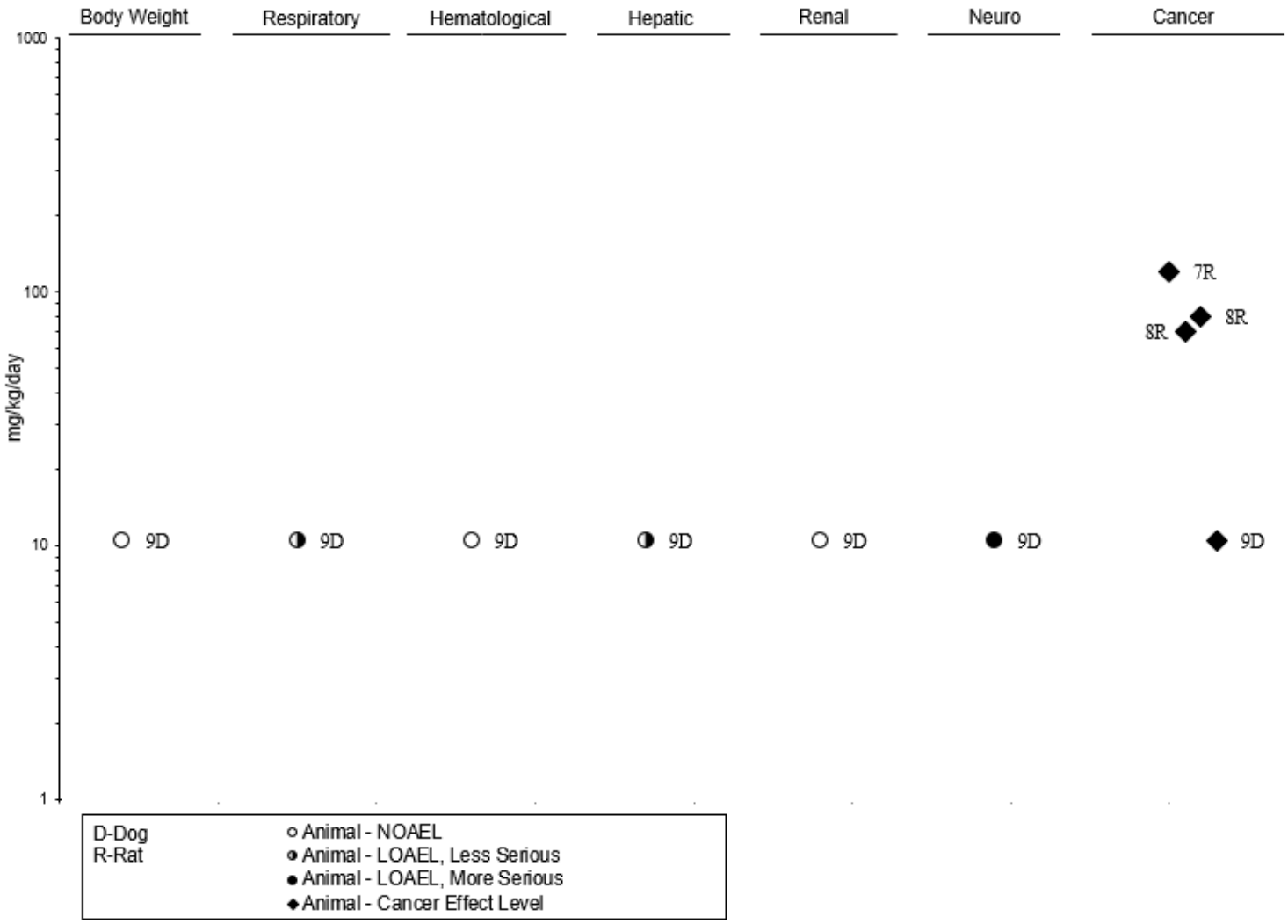


Figure 2-2. Levels of Significant Exposure to 3,3'-Dichlorobenzidine – Oral

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.