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 of 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.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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 first by route of exposure-inhalation, oral, and dermal; and then by health effect--death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure 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-observedadverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point 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 end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The

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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.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of 3,3'-dichlorobenzidine are indicated in Table 2- 1 and Figure 2- 1. Because cancer effects could occur at lower exposure levels, Figure 2-1 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA. A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure.

2.2.1 Inhalation Exposure

3,3'-Dichlorobenzidine is not a volatile chemical. In the air, it may exist as dust particles or bound to particulate matter. The absorption of 3,3'-dichlorobenzidine from such respirable particles into the body depends, in part, on the size of the particle. Large particles tend to deposit in the upper airways and are subsequently cleared by ciliary action with little absorption across lung tissues. However, the ciliary action transports the particles to the epiglottis where they are often swallowed, leading to gastrointestinal absorption. Smaller particles can penetrate more deeply into the respiratory tree, where 3,3'-dichlorobenzidine absorption may be significant.

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2.2.1.1 Death

No studies were located regarding lethal effects in humans or animals after inhalation exposure to 3,3'-dichlorobenzidine. No fatalities were observed in rats observed for 14 days following a l-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 per day for 7 days (Gerarde and Gerarde 1974).

2.2.1.2 Systemic Effects

No studies were located regarding cardiovascular, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, body weight, or metabolic effects in humans or animals after inhalation exposure to 3.3'-dichlorobenzidine.

Respiratory Effects. Upper respiratory infection and sore throat were listed among several principal reasons for visits to a company's medical clinic by workers handling 3,3'-dichlorobenzidine dihydrochloride (Gerarde and Gerarde 1974). However, there is no conclusive evidence that these effects were due to inhalation of 3,3'-dichlorobenzidine dihydrochloride.

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 per 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.

Gastrointestinal Effects. Gastrointestinal upset was one of the symptoms reported by employees who worked with 3,3'-dichlorobenzidine dihydrochloride (dihydro salt of 3,3'-dichlorobenzidine) (Gerarde and Gerarde 1974). However, there is no conclusive evidence that the gastrointestinal effects, or other symptoms reported by employees, resulted specifically from inhalation of 3,3'-dichlorobenzidine dihydrochloride.

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No studies were located regarding gastrointestinal effects in animals following inhalation exposure to 3,3'-dichlorobenzidine.

2.2.1.3 Immunological and Lymphoreticular Effects

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

2.2.1.4 Neurological Effects

The only relevant information regarding neurological effects in humans exposed to 3,3'-dichlorobenzidine was found in an early study which reported that headache and dizziness were among several principal reasons why employees working with 3,3'-dichlorobenzidine in a chemical manufacturing plant visited the company medical clinic (Gerarde and Gerarde 1974). However, there is no conclusive evidence that these symptoms were caused specifically by 3,3'-dichlorobenzidine since there was exposure to other chemicals as well. No further information was provided.

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

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

2.2.1.5 Reproductive Effects
2.2.1.6 Developmental Effects
2.2.1.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

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2.2.1.8 Cancer

Several epidemiological studies have investigated cancer incidences among workers occupationally exposed to 3,3'-dichlorobenzidine (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Myslak et al. 1991). Exposure may have been by both inhalation and dermal routes.

Due, in part, to structure-activity considerations, epidemiological studies of potential cancer effects of occupational exposure to 3,3'-dichlorobenzidine have been particularly concerned with bladder tumors, since 3,3'-dichlorobenzidine is structurally similar to benzidine, a chemical which is known to be a human bladder carcinogen. The possible role of benzidine-based azodyes as a carcinogenic risk factor for painters in a major industrial area of Germany was investigated by Myslak et al. (1991). The cohort consisted of 403 male patients (case group) treated in the period 1984-1987 for urological tumors: 290 had a diagnosis of bladder carcinoma and 113 had a diagnosis of bladder papilloma. The mean duration of employment was 29 years (range 2-48 years). A comparison group (reference group) of 426 patients with benign prostate disease was also included in the study. Cases and controls responded to questionnaires regarding employment history. Questionnaires were analyzed for occupational categories. A painter was defined as a person employed in this occupation for at least 6 months at any time of his working history and who had never been employed in another occupation known to be causally associated with bladder cancer. Of the bladder tumor patients, 21 were painters; among referents, 8 were painters. This difference among the groups was statistically significant; the relative risk of painters to be associated with bladder tumor was 2.76 (p<0.01). Occupation as painter (primarily house painter) was far more frequent among bladder tumor patients than would be expected from census data. The relative risk of bladder tumors for current smokers and ex-smokers was 1.13, which led Myslak et al. (1991) to suggest that the risk of smoking for bladder tumors was less than the occupational risk for the painters. The authors noted that a large number of benzidine-based azodyes were manufactured in Germany in the past. During that time it was usual for painters to prepare the paints themselves, allowing for possible exposure to dyes and pigments derived from benzidine, 3,3'-dichlorobenzidine, 3,3'-dimethylbenzidine (*o*-tolidine), 3,3'-dimethoxybenzidine (*o*-dianisidine), and 2-naphthylamine (Myslak et al. 1991). While the results of this study suggest that occupational exposure to benzidine-like chemicals is associated with an increased incidence in bladder tumors, the specific role of 3,3'-dichlorobenzidine, if any, is unknown.

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No other epidemiological studies have found either bladder tumors or excess tumors at other sites that were associated with 3,3'-dichlorobenzidine (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975). However, these studies were conducted with workers who were exposed to 3,3'-dichlorobenzidine for less than 20 years. Since a period of 5 to 50 years may follow the exposure to bladder carcinogens and the diagnosis of bladder cancer by a physician (Badalament 1998), an adequate latency period for 3,3'-dichlorobenzidine-induced tumors may not have elapsed for some individuals. Also, the number of workers examined in these studies was relatively small, thus limiting the statistical power to detect a significant increase in bladder cancer mortality (incidence). Finally, the possibility that 3,3'-dichlorobenzidine is a human carcinogen under certain undefined exposure conditions cannot be totally ruled out.

In one of these reports, no bladder tumors were found in a group of 35 workers who handled only 3,3'-dichlorobenzidine; in the same dyestuff plant, bladder tumors occurred in 3 out of 14 workers exposed to both benzidine and 3,3'-dichlorobenzidine. The investigator reported a total exposure time of 68,505 hours, equivalent to nearly 140 full-time working years (Gadian 1975).

No cases of bladder tumors were found in an epidemiology study of 259 workers exposed to dry and sernidry 3,3'-dichlorobenzidine base and hydrochloride. Cytological analyses of the urine (Papanicolaou tests) were negative. Workers were exposed to an average of less than 16 years each to 3,3'-dichlorobenzidine, which means that an adequate exposure duration and/or the latent period following exposure may not have been reached for tumor expression (MacIntyre 1975).

In a retrospective epidemiological study of workers employed in a dye and pigment manufacturing plant that used 3,3'-dichlorobenzidine as chemical precursor, no bladder tumors were observed in a cohort of 207 workers, most of whom had been exposed for up to 15 years (Gerarde and Gerarde 1974). However, in this study there was no evidence that any valid system of medical surveillance of workers ever existed during the years that 3,3'-dichlorobenzidine was used at the plant. A number of employees had not been followed up for 15 years or more (Gerarde and Gerarde 1974). Other limitations of this study included using data from a very small and incomplete sample of workers; focusing solely on the occurrence of bladder tumors; and using data that may have been misleading and, at times, apparently inaccurate.

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No studies were located regarding cancer effects in animals after inhalation exposure to 3,3'-dichlorobenzidine. However, cancer effects have been observed in animal studies where 3,3'-dichlorobenzidine was administered orally or by other routes. See Sections 2.2.2.8 and 2.5 for further information.

2.2.2 Oral Exposure

Indirect gastrointestinal tract exposure may occur from breathing contaminated airborne dust in the workplace. The respiratory deposition pattern of inhaled 3,3'-dichlorobenzidine depends primarily on the mass median aerodynamic diameter (MMAD) of the particles. The mucociliary clearance mechanism moves most particulates with a MMAD of l-5 μm out of the lower respiratory tract, thus allowing their passage into the gastrointestinal tract. Larger particles (>5 μm) impacting in the nasopharyngeal region would also be eventually ingested. Oral exposure may potentially occur in the general environment by drinking contaminated groundwater. Occupational exposure by the oral route is not expected to be significant. Exposure through eating food is unlikely since 3,3'-dichlorobenzidine has never had an application as an agricultural or food chemical. Children may be exposed to 3,3'-dichlorobenzidine if they consume contaminated soil; however, the bioavailability of 3,3'-dichlorobenzidine from soil is quite low. All of the available data on the effects of 3,3'-dichlorobenzidine following oral exposure are derived from studies in experimental animals. Table 2- 1 and Figure 2- 1 summarize available data.

2.2.2.1 Death

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

In rats, the acute-duration oral LD_{50} (lethal dose, 50% kill) 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. Both oral LD_{50} values for 3,3'-dichlorobenzidine are shown in Table 2-1 and plotted in Figure 2-l.

Table 2-1. Levels of Significant Exposure to 3,3'-Dichlorobenzidine · Oral

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Death							
1	Rat (albino)	once (GO)				7070 (LD$_{50}$)	Gerarde and Gerarde 1974 3,3-dichloro-benzidine base
2	Rat (Sprague-Dawley)	once (GO)				3820 (LD$_{50}$)	Gerarde and Gerarde 1974 3,3-dichloro-benzidine dihydrochloride
Systemic							
3	Rat (Wistar)	once (GO)	Hemato		127 F (hemoglobin adduction)		Birner et al. 1990 3,3-dichloro-benzidine dihydrochloride
INTERMEDIATE EXPOSURE							
Cancer							
4	Mouse (ICR)	6 or 12 mo (F)				170 M (hepatomas in 8/8 at 6 mo and in 18/18 at 12 mo)	Osanai 1976
5	Mouse (Strain D)	10 mo (F)				11.2-11.9 (hepatic tumors in 4/18)	Pliss 1959

Table 2-1. Levels of Significant Exposure to 3,3'-Dichlorobenzidine - Oral (continued)

| Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
CHRONIC EXPOSURE							
Systemic							
6	Dog (Beagle)	3 x/wk 6 wk + 5 x/wk 7.1 yr (C)	Resp		10.4 F (dyspnea in 1/6)		Stula et al. 1978
			Hemato	10.4 F			
			Hepatic		10.4 F (increased plasma GPT levels; fatty changes in liver in 1/6)		
			Renal	10.4 F			
			Body Wt	10.4 F			
Neurological							
7	Dog (Beagle)	3 x/wk 6 wk + 5 x/wk 7.1 yr (C)				10.4 F (convulsions and slight neuronal degeneration in 1/6 dogs)	Stula et al. 1978

Table 2-1. Levels of Significant Exposure to 3,3'-Dichlorobenzidine - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	

Cancer

8	Rat (Rappolovskii)	12 mo 6 d/wk (F)				120 (tumors in Zymbal gland, skin, mammary gland, ileum, bladder, hemopoetic, connective tissue, salivary gland, liver, thyroid)	Pliss 1959
9	Rat (Sprague-Dawley)	16 mo ad lib (F)				70 M (CEL: malignant mammary gland adeno- carcinomas in 7/44; Zymbal gland squamous cell carcinomas in 8/44; granulocytic leukemia in 9/44)	Stula et al. 1975
						80 F (CEL: malignant mammary gland adenocarcinomas in 26/44 females)	
10	Hamster (Golden	NS (F)				300 (transitional cell bladder carcinomas, liver-cell and cholangiomatous tumors)	Sellakumar et al. 1969

Table 2-1. Levels of Significant Exposure to 3,3'-Dichlorobenzidine ⁻ Oral (continued)

| Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
11	Dog (Beagle)	3x/wk 6 wks + 5x/wk 7.1 yrs (C)				10.4 F (CEL: hepatocellular carcinomas in 4/6, papillary transitional cell carcinomas of urinary bladder in 5/6)	Stula et al. 1978

[a]The number corresponds to entries in Figure 2-1.

ad lib = ad libitum; Body Wt = body weight; (C) = capsule; CEL = cancer effect level; F = female; (F) = feed; (G) = gavage; (GO) = gavage in oil; GPT = glutamic pyruvic transaminase; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; mo = month(s); NOAEL = no-observable-adverse-effect level; NS = not specified; wk = week(s); x = times; yrs = years

Figure 2-1. Levels of Significant Exposure to 3,3-Dichlorobenzidine - Oral
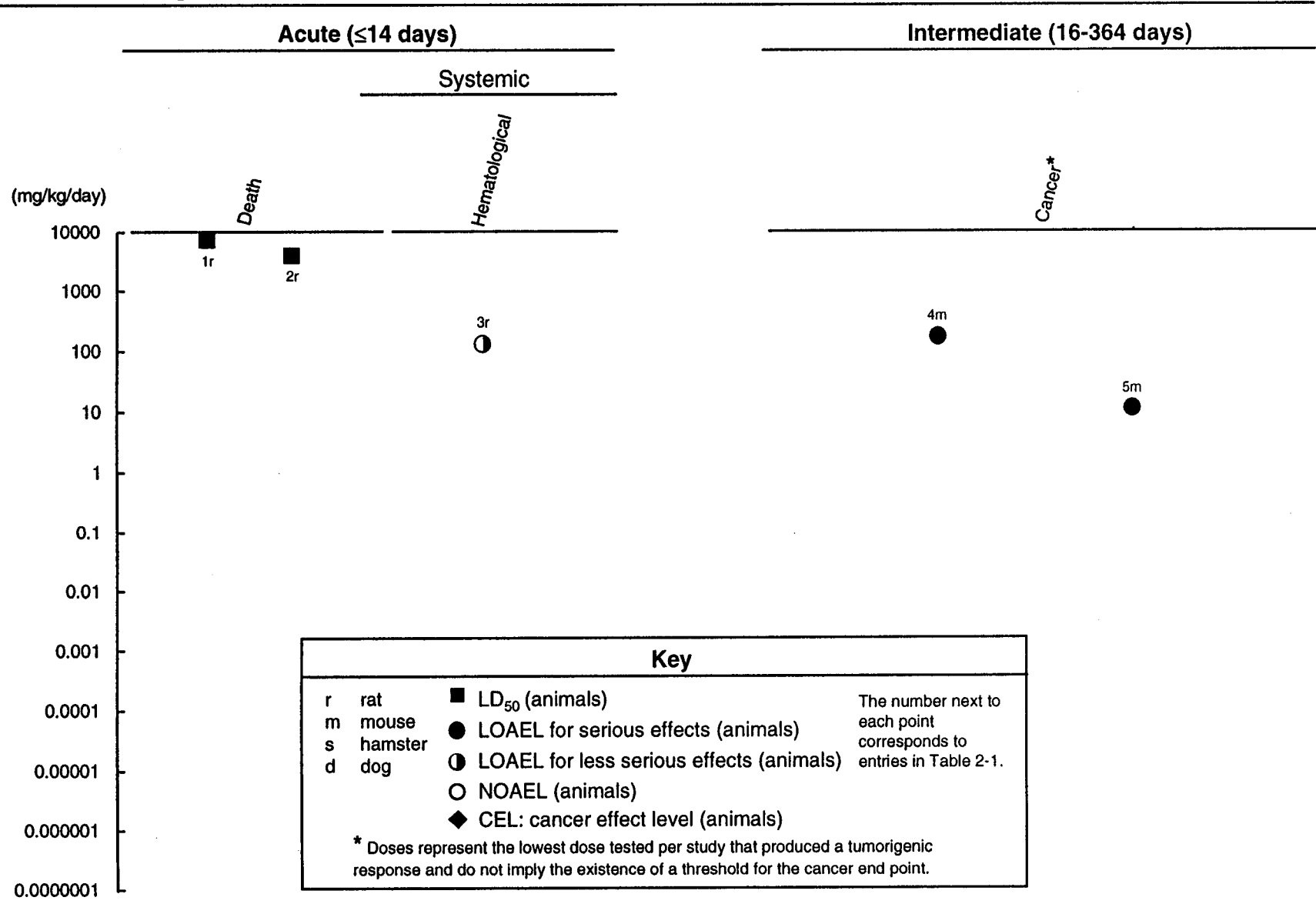
Figure 2-1. Levels of Significant Exposure to 3,3-Dichlorobenzidine - Oral (cont.)
Chronic (≥365 days)
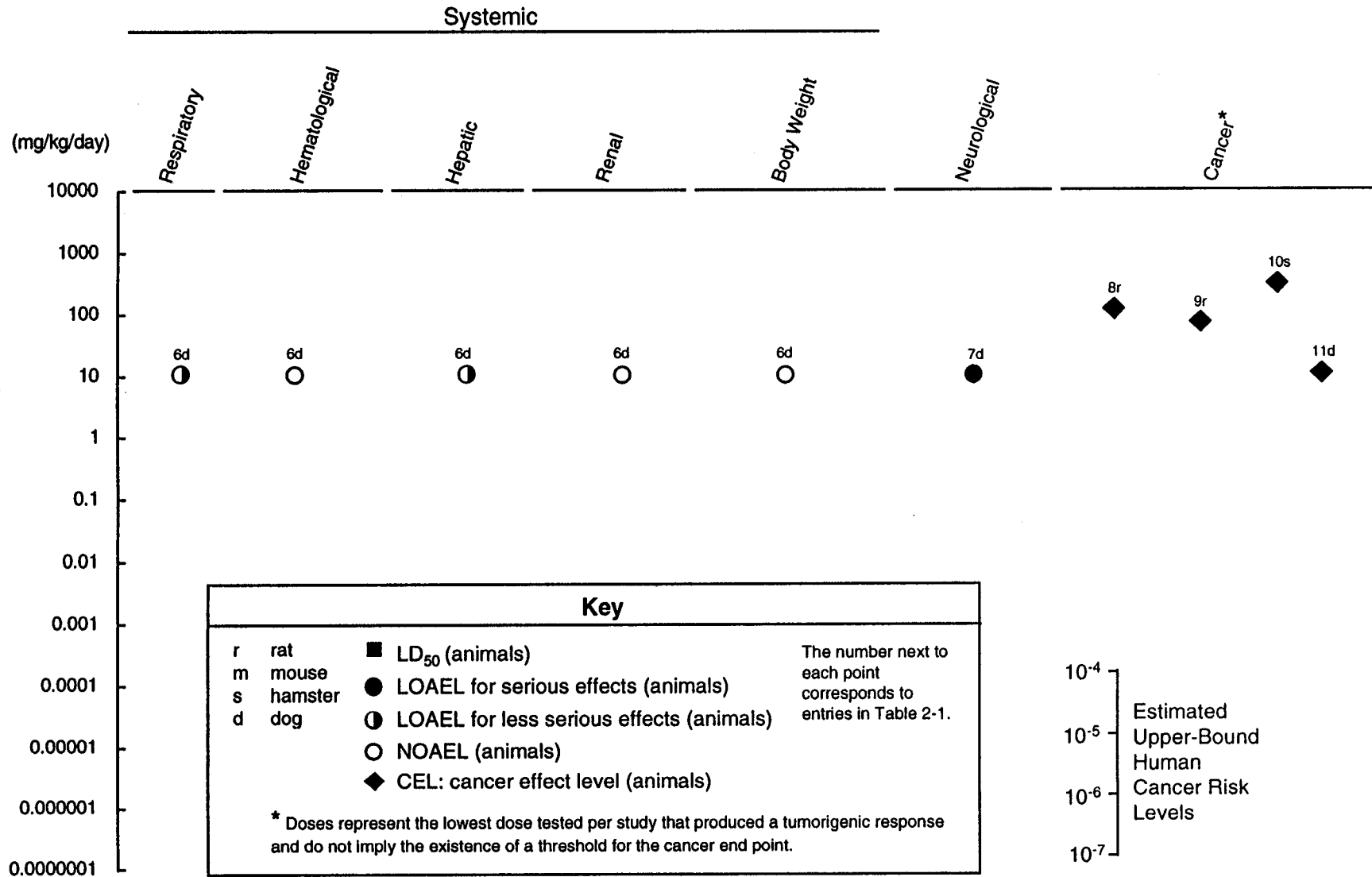
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2.2.2.2 Systemic Effects

No studies were located regarding respiratory, hematological, hepatic, renal, or body weight effects in humans after oral exposure to 3,3'-dichlorobenzidine. No studies were located regarding cardiovascular, gastrointestinal, musculoskeletal, endocrine, dermal, ocular, or metabolic effects in humans or animals after oral exposure to 3,3'-dichlorobenzidine.

The highest NOAEL values and all LOAEL values for oral exposure from each reliable study for systemic effects in each species and duration category for 3,3'-dichlorobenzidine are shown in Table 2-1 and plotted in Figure 2- 1.

Respiratory Effects. Dyspnea was observed in 1 of 6 female dogs exposed to 10.4 mg/kg/day 3,3'-dichlorobenzidine for 6.6 years, which probably 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).

Hematological Effects. Although hematological effects may not be sensitive indicators for 3,3'-dichlorobenzidine toxicity, 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) or with repeated doses between 0.3 and 5.8 mg/kg/day (Joppich-Kuhn et al. 1997). It was suggested that metabolically formed nitroso derivatives and the formation of a sulfinic acid amide with cysteine residues in hemoglobin may be the mechanism of adduct formation (Birner et al. 1990). Hydrolysis yielded mainly 3,3'-dichlorobenzidine; N-acetylated 3,3'-dichlorobenzidine was also detected. The more recent study found that adduct formation was dose-related (Joppich-Kuhn et al. 1997). It was further observed that at low doses of 3,3'-dichlorobenzidine, N-acetyl-3,3'-dichlorobenzidine adducts and 3,3'-dichlorobenzidine adducts were formed at similar levels, but at the highest dose level tested (5.8 mg/kg/day) the dichlorobenzidine adduct was predominant, suggesting saturation of the acetylation pathway at high dose (Joppich-Kuhn et al. 1997). While hemoglobin adduct formation does not imply altered or abnormal hemoglobin function, adduct formation may be a suitable biomarker of human exposure to 3,3'-dichlorobenzidine (see Section 2.7). Hematological variables (erythrocyte count, hemoglobin concentration, hematocrit, and leucocyte 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).

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Hepatic Effects. Limited animal evidence suggests that chronic-duration oral exposure to 3,3'-dichlorobenzidine results in mild-to-moderate liver injury. Six female dogs exposed to 3,3'-dichlorobenzidine (10.4 mg/kg/day) all had modestly elevated plasma glutamic-pyruvic transaminase (GPT) during the first 3 years of a 7-year treatment period (Stula et al. 1978). Thereafter, GPT levels returned to normal in three of the experimental animals, two remained elevated for the duration of the study. Elevated GPT levels may have been due to the test chemical that caused chronic hepatic injury to 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. It should be noted that the study is limited by use of one dose level, precluding dose-response evaluations. It should be mentioned, however, that none of the six control dogs exhibited adverse liver effects.

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

Body Weight Effects. In a study in which female dogs were exposed to 10.4 mg/kg/day 3,3'-dichlorobenzidine for 7 years, there were no significant differences in body weight between treated and control dogs during the study period (Stula et al. 1978).

2.2.2.3 Immunological and Lymphoreticular Effects

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

2.2.2.4 Neurological Effects

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

In a 3,3'-dichlorobenzidine carcinogenicity study, 1 of 6 dogs exhibited convulsions after 21, 28, or 42 months of oral treatment with 10.4 mg/kg/day over a period of 3.5 years (Stula et al. 1978). Necropsy

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at 42 months revealed slight neuronal degeneration; 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. This LOAEL value for neurological effect for oral exposure to 3,3'-dichlorobenzidine is shown in Table 2- 1 and plotted in Figure 2- 1.

2.2.2.5 Reproductive Effects

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

2.2.2.6 Developmental Effects

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

2.2.2.7 Genotoxic Effects

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

Genotoxic effects have been reported in animals treated with 3,3'-dichlorobenzidine. A single dose of 3,3'-dichlorobenzidine (1,000 mg/kg) administered to male and pregnant female mice induced micronuclei in polychromatic erythrocytes in the bone marrow of the males and in the liver of the fetuses, but not in bone marrow of the dams (Cihak and Vontorkova 1987). A micronucleus test is performed to detect a chemical's ability to induce chromosomal aberrations. However, the relevance of micronuclei formation to human health is not known. The reason for the lack of effect of 3,3'-dichlorobenzidine on bone marrow micronuclei formation in the mothers is unclear, but it may be related to deficiencies in the metabolic activation of 3,3'-dichlorobenzidine in female mice. The relative importance of pregnancy is unknown since the study did not evaluate nonpregnant females. In another study, an increase in unscheduled deoxyribonucleic acid synthesis (UDS) was observed in cultured liver cells from male mice previously pretreated orally with single doses of ≥ 500 mg/kg 3,3'-dichlorobenzidine; no response was observed at a dose of ≤ 200 mg/kg (Ashby and Mohammed 1988).

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3,3'-Dichlorobenzidine was also shown to bind extensively to tissue deoxyribonucleic acid (DNA) in rats and mice. Single oral administration of 20 or 100 mg/kg radiolabeled 3,3'-dichlorobenzidine to male Sprague-Dawley rats and Swiss-Webster mice resulted in extensive binding of the compound to tissue (liver, bladder, and intestine) DNA at 12, 24, or 96 hours, and 9 or 14 days after treatment (Ghosal and Iba 1990).

The UDS assay is used to measure the repair that follows DNA damage. However, the relevance of UDS to human health is not known. While results were positive in two assay in animals, sufficient data are not available from more predictive indicator assays to adequately characterize the genotoxic potential for 3,3'-dichlorobenzidine in humans. Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

There are no epidemiological studies linking cancer in humans to oral exposure to 3,3'-dichlorobenzidine. However, based on the findings of oral studies in animals, 3,3'-dichlorobenzidine may be regarded as a chemical that would probably induce cancer in humans given sufficient exposure to the agent. An IARC review of the existing cancer toxicity data for 3,3'-dichlorobenzidine concluded that, although no case report on exposure to 3,3'-dichlorobenzidine was available, because 3,3'-dichlorobenzidine and benzidine may be made in the same plant, it is not possible to exclude 3,3'-dichlorobenzidine's contribution to the incidence of bladder cancer attributed to benzidine (IARC 1982a). Studies in animals demonstrated that 3,3'-dichlorobenzidine is carcinogenic in rats, hamsters, mice and dogs (see below).

A statistically significant increased incidence of hepatomas was observed in male ICR/JCL mice exposed to 0.1% 3,3'-dichlorobenzidine in the diet (170 mg/kg/day) at 6 months (8 of 8 treated as opposed to 0 of 5 controls) and 12 months (18 of 18 treated as opposed to 2 of 2 1 controls) (Osanai 1976). Hepatic tumors were observed in 4/l 8 strain D mice exposed to 11.2-l 1.9 mg 3,3'-dichlorobenzidine/kg/day in the diet for 10 months (Pliss 1959).

No bladder carcinomas were observed in rats exposed to 0.03% 3,3'-dichlorobenzidine in the diet (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 3,3'-dichlorobenzidine dihydrochloride/kg/day by gavage once every 3 days over a 30-day period and sacrificed 8 months later (Griswold et al. 1968).

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In a study in which rats were exposed to 10-20 mg 3,3'-dichlorobenzidine per day (120 mg/kg/day) in feed 6 days per week for 12 months, tumors were observed at a variety of sites, including the Zymbal gland (7 of 29 animals), mammary gland (7/29), bladder (3/29), hematopoietic system (3/29), skin (3/29), ileum (2/29), connective tissue (2/29), salivary gland (2/29), liver (l/29), and thyroid (l/29) (Pliss 1959). No tumors were reported in 130 control animals. In a later study, the same investigator reported that oral administration of an unspecified dose (in the range of 125-500 mg/kg) of 3,3'-dichlorobenzidine by gavage to rats for 10-13 months resulted in the development of tumors of the skin, sebaceous and mammary glands, and papillomas of the urinary bladder (Pliss 1963). Because the frequency of administration of the compound was not provided, a daily dose could not be estimated.

In another rat study, 3,3'-dichlorobenzidine was administered to 50 male (70 mg/kg/day) and 50 female (80 mg/kg/day) Sprague-Dawley rats, in a standard diet for up to 16 months (Stula et al. 1975). In rats fed 3,3'-dichlorobenzidine in the diet for a total of 349 days (females) and 353 days (males), histopathological evaluations revealed mammary adenocarcinoma (16% incidence), malignant lymphoma (14%) granulocytic leukemia (20%), carcinoma of the Zymbal gland (18%) in males, and mammary adenocarcinoma (59%) in females. These tumors were either totally absent or occurred statistically less frequently in untreated controls. The authors noted that most of these tumors appeared to arise in the bone marrow and hematopoietic foci in the spleen and liver with subsequent metastasis to other organs. Only one dose level was used in the study, however, and information on the purity of the test substance was not provided.

In a subsequent study by this investigator, hepatocellular carcinomas (67% incidence) and papillary transitional cell carcinomas of the urinary bladder (83%) were observed in female dogs fed approximately 10.4 mg/kg/day orally in gelatin capsules over a period of 6.6-7.1 years (Stula et al. 1978). These tumors were absent in untreated controls. 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 unequivocally the carcinogenicity of this chemical in this species.

Transitional cell bladder carcinomas and liver cell and cholangiomatous tumors were observed in hamsters fed a diet containing 0.3% 3,3'-dichlorobenzidine (300 mg/kg/day) (Sellakumar et al. 1969). This level was determined to be the maximum tolerated dose. In an earlier study, a diet containing 0.1% 3,3'-dichloro-

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benzidine (59-64 mg/kg/day) fed to Syrian golden hamsters for their lifetimes did not cause significant carcinogenic effects or changes in bladder pathology (Saffiotti et al. 1967).

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-(5nitro-2-furyl)-2-thiazolyllformamide (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'-dichlorobenzidine had a synergistic effect on the carcinogenicity of BBN. In rats sequentially administered BBN (0.0l%), 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 4 chemicals was no different than after administration of the first 3, suggesting no interactive effect of any type for 3,3'-dichlorobenzidine (Ito et al. 1983).

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-l and plotted in Figure 2-l. Based on the increased incidence in mammary adenocarcinomas in rats reported in the Stula et al. (1975) study, EPA calculated a q_1* of 0.45 $(mg/kg/day)^{-1}$. Doses corresponding to risk levels ranging from 10^{-4} to 10^{-7} are 2.2×10^{-4} to 2.2×10^{-7} mg/kg/day, respectively, as indicated in Figure 2-l.

2.2.3 Dermal Exposure

Because of large particle size and increased usage of closed systems and protective clothing, dermal absorption is expected to be minimal in occupational environments. Conditions of high humidity and high temperature are known to enhance dermal absorption of chemicals following skin contact.

2.2.3.1 Death

No studies were located regarding lethal effects in humans after dermal exposure to 3,3'-dichlorobenzidine. The minimum dermal lethal dose for 3,3'-dichlorobenzidine (free base) for male and female New Zealand

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albino rabbits with skin intact was reported to be greater than 8,000 mg/kg (Gerarde and Gerarde 1974). The cause of death was not discussed. No discernible skin irritation was observed when 3,3'-dichlorobenzidine dihydrochloride was applied to the intact or abraded skin of rabbits; the dose was not provided (Gerarde and Gerarde 1974). This minimum dermal lethal dose in female New Zealand albino rabbits is shown in Table 2-2. Dermal exposure is not likely to cause death in humans.

2.2.3.2 Systemic Effects

No information was located regarding cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, body weight, or metabolic effects in animals or humans following dermal exposure to 3,3'-dichlorobenzidine.

Very limited data were found regarding the effects of dermal exposure to 3,3'-dichlorobenzidine. The highest NOAEL value and all LOAEL values for dermal exposure for this study are shown in Table 2-2.

Respiratory Effects. Although no respiratory effects have been reported in humans following dermal exposure exclusively to 3,3'-dichlorobenzidine, upper respiratory infection and sore throat were among the principal reasons for visits to a company's medical clinic by workers who handled 3,3'-dichlorobenzidine (Gerarde and Gerarde 1974). However, there is no conclusive evidence that these effects were due specifically to 3,3'-dichlorobenzidine exposure. Workers may have been exposed to this and/or other agents by both inhalation and dermal routes.

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

Dermal Effects. Dermatitis was cited as the only verified health problem encountered by workers in contact with the free base of 3,3'-dichlorobenzidine in a dichlorobenzidine manufacturing plant (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

Table 2-2. Levels of Significant Exposure to 3,3'-Dichlorobenzidine - Dermal

| Species (Strain) | Exposure/ Duration/ Frequency | System | NOAEL | LOAEL | | Reference Chemical Form |
				Less serious	Serious	
ACUTE EXPOSURE						
Death						
Rabbit (New Zealand)	NS				>8000 mg/kg (minimum lethal dose)	Gerarde and Gerarde 1974 3,3-dichloro-benzidine base
Systemic						
Rabbit (NS)	NS	Ocular	100 mg			Gerarde and Gerarde 1974 3,3-dichloro-benzidine base
Rabbit (NS)	NS	Ocular		0.1 mL (erythema, pus, and opacity)		Gerarde and Gerarde 1974 3,3-dichloro-benzidine dihydrochloride

LOAEL = lowest-observable-adverse-effect level; NOAEL = no-observable-adverse-effect level; NS = not specified

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).

Ocular Effects. No studies were located regarding the ocular effects of 3,3'-dichlorobenzidine in humans.

No effects were reported in rabbits when 100 mg of dichlorobenzidine (free base) was placed in the conjunctival sac of the eye (Gerarde and Gerarde 1974). It should be noted that the authors did not report the duration of exposure or the vehicle used. However, 0.1 mL of 3,3'-dichlorobenzidine dihydrochloride in a 20% corn oil suspension produced erythema, pus, and cornea1 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 very likely associated with the release of hydrochloric acid following the salt's contact with the moist surface of the eye.

No studies were located regarding the following effects in humans or animals after dermal exposure to 3.3'-dichlorobenzidine:

2.2.3.3 Immunological and Lymphoreticular Effects
2.2.3.4 Neurological Effects
2.2.3.5 Reproductive Effects
2.2.3.6 Developmental Effects
2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

Several epidemiological studies have investigated cancer incidences among workers occupationally exposed to 3,3'-dichlorobenzidine and other arylamines (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Myslak et al. 1991). Exposure may have been by both inhalation and dermal routes. These studies are discussed in greater detail under Section 2.2.1.8 (inhalation cancer effects).

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Due, in part, to structure-activity considerations, epidemiological studies of potential cancer effects of occupational exposure to 3,3'-dichlorobenzidine have been particularly concerned with bladder tumors since benzidine is a known human carcinogen in which the bladder is the primary target. While one study found an excess incidence of bladder tumors among German painters who may have been exposed to 3,3'-dichlorobenzidine (Myslak et al. 1991), the causality is equivocal largely because it had been common for painters to prepare the paints themselves, allowing for possible exposure to other carcinogenic dyes and pigments derived from benzidine, 3,3'-dichlorobenzidine, 3,3'-dimethylbenzidine (o-toluidine), 3,3'-dimethoxybenzidine (*o*-dianisidine), and 2-naphthylamine (Myslak et al. 1991).

A more recent study found an association between bladder cancer and exposure to arylamines (Ouellet-Hellstrom and Rench 1996). This study examined the cancer incidence in a cohort of 704 workers employed at a Connecticut chemical plant between 1965 and 1989. The plant produced a variety of chemicals including arylamines such as 3,3'-dichlorobenzidine, o-tolidine, and o-dianisidine, but not benzidine; benzidine production ceased prior to mid-1965. Skin contact was found to be the main route of exposure. Only workers never exposed to benzidine were selected to participate and only confirmed cancer cases were considered in the analysis. As a result of a worker survey, the information on follow-up yielded 8,624 person-years of observation for a follow-up rate of 97% for male employees and 1,660 person-years for a follow-up rate of 97% for female employees. There were a total of 27 cancer cases, 23 in males and 4 in females. Three of the 23 male cases were non-melanoma skin cancers and were not included in the analysis. There were 7 cases of bladder cancer, all in males; two were diagnosed in workers first employed after 1972, four in workers first employed at the age of 40 or older, and five in workers who worked at least 5 years or more. All bladder cancers had a follow-up period of 8 years or more. The standardized incidence ratio (observed/expected, SIR) for bladder cancer was 8.3 (C.I. 3.3-17.0). In addition, the association between bladder cancer cases and exposure to arylamines increased with cumulative exposure. One bladder cancer case was a current smoker and the other six were former smokers. The authors (Ouellet-Hellstrom and Rench 1996) recognized that the study could not evaluate cancer risks for specific arylamines, but as indicated above, the results supported an association between bladder cancer and arylamine exposure. They also indicated that although smoking is known to increase the risk of bladder cancer by a factor of two, it is unlikely that smoking alone explains the eight-fold increase in bladder cancer risk observed in the study.

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No studies were located regarding carcinogenicity in animals following dermal exposure to
3,3'-dichlorobenzidine.

2.3 TOXICOKINETICS

Very 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. Evidence from animal studies suggest 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). 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.

2.3.1   Absorption

There is no information regarding absorption of 3,3'-dichlorobenzidine in children by any route
of exposure.

2.3.1.1 Inhalation Exposure

3,3'-Dichlorobenzidine has been detected in the urine of workers in 3,3'-dichlorobenzidine-
handling plants under conditions which favored inhalation of 3,3'-dichlorobenzidine-bound
particulate matter (Handke et al. 1986; London and Boiano 1986; Meigs et al. 1954). Under these
conditions, it is reasonable to expect that some of the 3,3'-dichlorobenzidine found in the urine
could have come from inhalation exposure. However, conditions in the plants were also
conducive to dermal exposure. Therefore, some of the 3,3'-dichlorobenzidine dose found in the
urine could have come from dermal exposure. In addition, since the mucocilliary clearance
mechanism moves most of the 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. No information was located on absorption in animals following inhalation exposure.

2.3.1.2 Oral Exposure

No quantitative data were located on the absorption of 3,3'-dichlorobenzidine following oral exposure in humans. However, a study in volunteers found acetylated metabolites in the urine 24 hours after a single 250 mg oral dose of 3,3'-dichlorobenzidine, which suggested that the compound is absorbed (Belman et al. 1968).

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, indicating a high bioavailability, typical of primary arylamines. The elimination is biphasic, with half-lives of 6 hours and 14 hours in plasma for the rapid and slow phases, respectively (Hsu and Sikka 1982).

2.3.1.3 Dermal Exposure

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 is minimized. 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).

2.3.2 Distribution

There is no information regarding distribution of 3,3'-dichlorobenzidine or metabolites in children after exposure by any route.

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2.3.2.1 Inhalation Exposure

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

2.3.2.2 Oral Exposure

No studies were located regarding distribution of 3,3'-dichlorobenzidine in humans after oral exposure.

In animals, orally absorbed 3,3'-dichlorobenzidine is widely distributed. In a study in which 3,3'-dichlorobenzidine was orally administered to female Wistar rats in single doses of 0.25 mL 3,3'-dichlorobenzidine in propylene glycol at 0.5 or 1 mmol/kg (127 or 253 mg/kg) by gavage, hemoglobin adducts of 3,3'-dichlorobenzidine were isolated from the blood of the animals (Birner et al. 1990). Similar results were obtained in rats dosed with 0.3-5.8 mg 3,3'-dichlorobenzidine/kg/day for 4 weeks (Joppich-Kuhn et al. 1997). The distribution of radioactivity in rat tissues after the oral administration of [^{14}C]-3,3'-dichlorobenzidine has been studied (Hsu and Sikka 1982). Twenty-four hours after a single oral dose, the highest levels of radioactivity were found in the liver, followed by the kidney, lung, spleen, heart, pancreas, and testes, in that order. This pattern did not depend on dose. After 96 hours, tissues that retained 0.02% or more of the administered radioactivity were liver (1.48%), muscle (0.37%), kidney (0.19%), and lung (0.02%). Erythrocytes retained more of the radioactivity than lung, but attention was not paid to the hematopoietic system in this study (Hsu and Sikka 1982). The effect of repetitive 3,3'-dichlorobenzidine administration on tissue levels of radioactivity was also studied by Hsu and Sikka (1982). Radioactivity in tissues of animals that received six daily doses of 3,3'-dichlorobenzidine was generally three to four 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 of the compound. 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). Overall, bioaccumulation of this chemical in rats is considered to be minimal following oral exposure of any duration.

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There is indirect evidence that 3,3'-dichlorobenzidine or 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 1967). There is no information regarding accumulation of 3,3'-dichlorobenzidine or metabolites in breast milk or its potential transfer to offspring via breast milk.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution of 3,3'-dichlorobenzidine in humans following dermal exposure. The distribution of [^{14}C]-3,3'-dichlorobenzidine in rat tissues following dermal application 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. 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 persistence exposure.

2.3.3 Metabolism

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

Information from a study in which 4 volunteers ingested a single 250 mg dose of 3,3'-dichlorobenzidine 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 (Lazear et al. 1979; Reid et al. 1984; Tanaka 1981). 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 5 metabolites of 3,3'-dichlorobenzidine in addition to the parent compound. None of the metabolites were identified, but a majority were reported to be conjugates (Hsu and Sikka 1982). A 24-hour urine sample of rats given a

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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). 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 (Bimer et al. 1990). Hemoglobin adducts were detected by the release of 3,3'-dichlorobenzidine after alkaline hydrolysis. 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 sulfhydryls in cysteine residues of hemoglobin.

No studies were located regarding the metabolism of 3,3'-dichlorobenzidine in humans following dermal exposure. 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 utagenicity of diacetylated product is much less than either the monoacetylated or parent compound (Lazear et al. 1979; Reid et al. 1984; Tanaka 1981), diacetylation may be a detoxification reaction for 3,3'-dichlorobenzidine (see also Sections 2.4.1 and 2.4.2).

There is no information regarding the metabolism of 3,3'-dichlorobenzidine in children. However, N-acetylation (as discussed above) in humans is likely done by one of two families of N-acetyltransferases. One of these families, NAT2, is developmentally regulated (Leeder and Keams 1997). Some enzyme activity can be detected in the fetus by the end of the first trimester. Almost all infants exhibit the slow acetylator phenotype between birth and 2 months of age. The adult phenotype distribution is reached by the age of 4-6 months, whereas adult activity is found by approximately l-3 years of age. Also, UDPglucuronosyltransferase, responsible for the formation of glucuronide conjugates, seems to achieve adult activity by 6-18 months of age (Leeder and Kearns 1997). These data suggest that metabolism of 3,3'-dichlorobenzidine by infants will differ from that in adults in extent, rate, or both.

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

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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 (Hoffman and Schmidt 1993; Leuschner 1978; NCTR 1979; Nony et al. 1980).

2.3.4 Elimination and Excretion

There is no information regarding the elimination and excretion of 3,3'-dichlorobenzidine or metabolites in children following any route of exposure.

2.3.4.1 Inhalation Exposure

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 authors did not clearly identify specific pigments. While the authors did not report exposure route, it was presumed to have been by inhalation. Dermal exposure may have also occurred.

No studies were located regarding excretion in animals after inhalation exposure to 3,3'-dichlorobenzidine.

2.3.4.2 Oral Exposure

Very limited information was located regarding excretion of 3,3'-dichlorobenzidine and/or metabolites in humans after oral exposure. In 4 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'-dichlorobenzidinederived 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'-dichloro-

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benzidine (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. 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).

2.3.4.3 Dermal Exposure

No studies were located regarding the excretion of 3,3'-dichlorobenzidine in humans following

dermal exposure. Fecal excretion in rats at 24 hours following 3,3'-dichlorobenzidine 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.

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

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the

uptake and disposition of chemical substances to quantitatively describe the relationships among

critical biological processes (Krishnan et al. 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 substance 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 end points.

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PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.
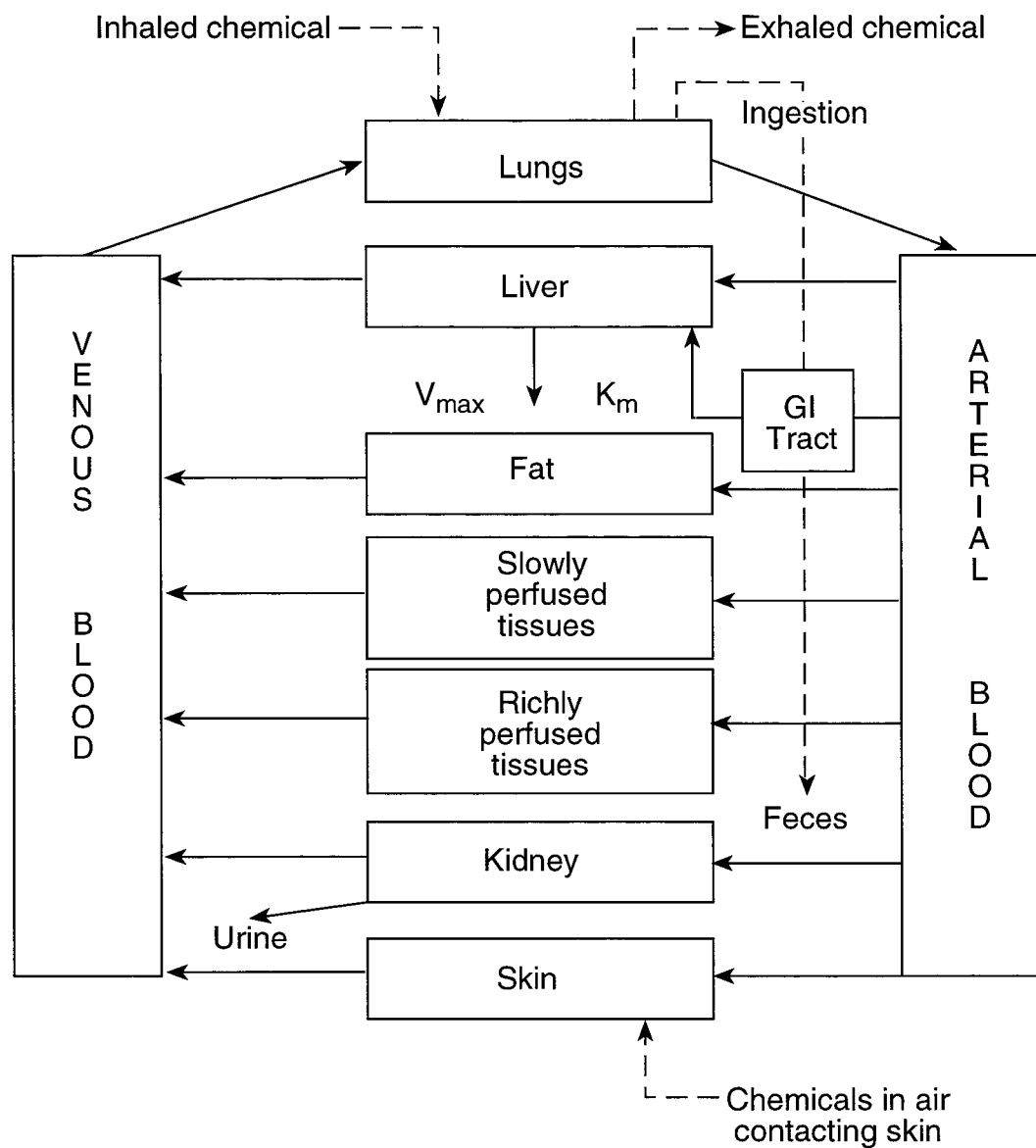
The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 2-2 shows a conceptualized representation of a PBPK model. If PBPK models for 3,3'-dichloro

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Figure 2-2. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance

Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

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benzidine exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

No PBPK modeling studies were located for 3,3'-dichlorobenzidine.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

No information was located for the mechanism of inhalation, oral, or dermal absorption of 3,3'-dichlorobenzidine in humans or animals. Also, no information was located for the mechanism by which 3,3'-dichlorobenzidine is transported in the blood. However, a studies in rats have shown that 3,3'-dichlorobenzidine forms adducts with hemoglobin (Birner et al. 1990; Joppich-Kuhn et al. 1997), indicating that at least a small amount of the chemical is associated with red blood cells.

3,3'-Dichlorobenzidine induces liver microsomal enzymes in a pattern similar to 3-methylcholanthrene. Liver microsomes from male Sprague-Dawley rats pretreated intraperitoneally with 3,3'-dichlorobenzidine yielded information to suggest that the induction pattern of P-450 isozymes by 3,3'-dichlorobenzidine resembles that of 3-methylcholanthrene. 3,3'-Dichlorobenzidine significantly induced ethoxy-coumarin O-deethylase, p-nitrophenetole O-deethylase, and arylhydrocarbon hydrolase by 5-, 6-, and 5-fold, respectively (Iba et al. 1983). Another study also found that 3,3'-dichlorobenzidine induces P-450 isozymes in a pattern similar to 3-methylcholanthrene (i.e., induces P-450c) (CYP2B l), and P-450d (CYPlA2) but mainly P-450c (CYP2Bl) (Iba and Thomas 1988). The same authors also conducted studies to identify the isozymes involved in NADPH-dependent activation of 3,3'-dichlorobenzidine by rat hepatic microsomes to mutagens in the Ames test. 3,3'-Dichlorobenzidine activation was unaffected by monoclonal antibodies to P-450b (CYPIAI) or P-450c (CYP2Bl) but was inhibited by 69% by polyclonal antibodies to P-450d (CYPlA2). 3,3'-Dichlorobenzidine activation was also inhibited 46% by antibody specific to NADPH-cytochrome P-450 reductase. Also, addition of methimazole, a high affinity substrate for the flavin-containing monooxygenase, reduced the residual mutagenicity in the systems containing antibody to P-450b (CYPlA2) and cytochrome P-450 reductase to 9% and 19%, respectively, of the appropriate control values. Based on these results, Iba and Thomas (1983) concluded that P-450d

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(CYPlA2) contributes to the majority of the P-450-dependent activation of 3,3'-dichlorobenzidine in hepatic microsomes.

If 3,3'-dichlorobenzidine is activated to a mutagenic intermediate by CYPlA2, this would have relevance to exposure *in utero* and in neonates. Human fetal liver does not contain appreciable amounts of CYPlA2 (Leeder and Kearns 1997). Adult levels of CYPlA2 are reached at about 4 months of age and may be exceeded in 1-2-year-old children. CYPlA2 levels subsequently decline and reach adult levels at the end of puberty.

2.4.2 Mechanisms of Toxicity

Although, data from the existing human and animal studies indicate that 3,3'-dichlorobenzidine is minimally toxic, its mechanism of toxicity appears to be well defined, deriving mainly from adduction of DNA. The available data suggest that the metabolism of 3,3'-dichlorobenzidine begins with the formation of nitroso derivatives which yield a sulfinic acid amide with hemoglobin in erythrocytes. This has been suggested to be a mechanism for adduct formation. However, *N*-oxidation at one of the two nitrogens could occur in the parent diamine, the monoacetyl, or the diacetyl derivative. *N*-hydroxy-dichlorobenzidine and *N*-hydroxyhr-acetyl-dichlorobenzidine could arise from either direct *N*-oxidation of the amino group or by deacetylation of the hydroxamic acid. Peroxidative activation of 3,3'-dichlorobenzidine will yield 3,3'-dichlorobenzidine diimine which causes DNA damage in bladder which might be responsible for tumor formation in this target in dogs and possibly humans. In rodents, *N*-oxidation of the monoacetyl derivative is an important step of metabolic activation (Birner et al. 1990).

Results from a recent study suggest that cytochrome P-450 (specifically CYP4B 1) activity may contribute to the initiation of carcinogenesis in rat and mouse bladder by activation of 3,3'-dichlorobenzidine to mutagenic compounds (Imaoka et al. 1997). The authors demonstrated the presence of CYP4B 1 in rat and mouse bladder microsomes by immunoblotting and immunohistochemistry. Furthermore, tissue-staining showed that CYP4Bl was present in epithelial cells of the bladder. It was also shown in that study that mouse bladder microsomes activated 3,3'-dichlorobenzidine, although not to the degree observed with renal microsomes and purified CYP4B 1. 3,3'-Dichlorobenzidine activation was judged by a gene expression test in *Salmonella typhimurium* NM2009 that detects DNA damage. Rat CYP4Bl produced very high mutagenic activity for 3,3'-dichlorobenzidine.

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The genotoxicity of 3,3'-dichlorobenzidine is derived from DNA adduction, as suggested by positivereverse mutation results in *Salmonella typhimurium* TA98 strain, since this strain of *S. typhimurium* detects reverse (histidine revertants) mutation in both activated and direct-acting base-pair substitution and frameship mutagens (Vithayathil et al. 1983). The extent of covalent binding of a compound to DNA and the persistence of the resulting adducts are considered important determinants of cancer initiation by genotoxic carcinogens (Ghosal and Iba 1990). As a direct-acting mutagen, 3,3'-dichlorobenzidine is an effective inducer of its own activation (Iba 1987a).

It has been suggested that some of the toxicity (carcinogenicity and non-cancer) of polyhalogenated aromatics (such as 3,3'-dichlorobenzidine) may be related to the abilities to induce cytochrome P-448-mediated (CYPlA2) monooxygenase activities. Therefore, it is reasonable to expect that the hepatocarcinogenicity of 3,3'-dichlorobenzidine may be due, at least in part, to the induction of hepatic cytochrome P-448 which would have the impact of producing higher amounts of reactive metabolites (Iba et al. 1983). The demonstration that 3,3'-dichlorobenzidine both increases lipid peroxidation and decreases antioxidant content *in vivo* in one study may have a bearing on the carcinogenicity of this substance because antioxidants protect against the acute and long-term effects of lipid peroxidation (Iba 1987b) which may be an important determinant in carcinogenesis.

There are data to suggest that 3,3'-dichlorobenzidine may act synergistically with other carcinogens. No carcinomas were found in any rats administered one of the following in the diet for a period of 40 weeks: 0.03% 3,3'-dichlorobenzidine in the diet, 0.001% BBN in drinking water, 0.0005% 2-AAF in the diet, or 0.04% FANFT (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. The authors suggested a synergistic effect of 3,3'-dichlorobenzidine on the carcinogenicity of BBN.

2.4.3 Animal-to-Human Extrapolations

Information on the toxicity of 3,3'-dichlorobenzidine for humans and animals is limited, particularly regarding noncancer end points. Therefore, an attempt to discuss potential interspecies differences or similarities in 3,3'-dichlorobenzidine noncancer toxicity based on the limited information available seems speculative at this time. 3,3'-Dichlorobenzidine is carcinogenic in animals (Osanai 1976; Pliss 1959, 1963;

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Sellakumar et al. 1969; Stula et al. 1975, 1978). There is no conclusive evidence of carcinogenicity of 3,3'-dichlorobenzidine in humans (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996); however, there is concern about occupationally exposed subjects because of 3,3'-dichlorobenzidine's structural similarity with the known human and animal carcinogen benzidine. However, unless a cohort exposed only to 3,3'-dichlorobenzidine is identified and adequate epidemiological studies on such a cohort are conducted, the question will remain unsolved.

2.5 RELEVANCE TO PUBLIC HEALTH

Overview.

Most of the information on human health effects of 3,3'-dichlorobenzidine is derived from several reports of exposure in the workplace, in which the inhalation and dermal routes represent the most likely routes of exposure. Significant exposure to 3,3'-dichlorobenzidine, would impact the health of the general population, seems unlikely. The available occupational studies have limitations, including lack of precise exposure data and presence of other compounds, as well as other confounding factors. No organ or system could be identified as a target for 3,3'-dichlorobenzidine toxicity in the available studies in humans. Results from cancer studies in humans were inconclusive because of possible co-exposure to other chemicals. Studies in animals showed that 3,3'-dichlorobenzidine is a multi-site carcinogen in various species following oral administration; no data were available following inhalation or dermal exposure. There is some evidence, however, of carcinogenicity in rats after subcutaneous injection of 3,3'-dichlorobenzidine, and in the offspring of mice after subcutaneous dosing to the dams during pregnancy. Systemic effects in animals were limited to reports of formation of adducts with proteins such as hemoglobin and with DNA and minor liver effects after chronic oral dosing. Also, ocular effects were reported in rabbits after direct instillation of the hydrochloric salt of the compound to the eye. In most studies in animals, the animals were exposed to levels of 3,3'-dichlorobenzidine several orders of magnitude higher than those found in the environment. Almost nothing is known about the toxicokinetics of 3,3'-dichlorobenzidine in humans. 3,3'-Dichlorobenzidine has been identified in the urine from workers or volunteers exposed to it; therefore, it is absorbed by humans. The primary route of absorption could not be ascertained, but it is assumed to have been inhalation and/or dermal. Animals can absorb 3,3'-dichlorobenzidine through ingestion or dermal contact with the chemical; no information was located regarding inhalation exposure. Based on limited data regarding environmental exposure, the most likely exposure route for populations

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living near hazardous waste sites is the dermal route. Under these circumstances, assuming that 3,3'-dichlorobenzidine is present in surrounding environmental media, this route may be of concern since animal studies have shown that 3,3'-dichlorobenzidine is absorbed by this route. Issues relevant to children are explicitly discussed in Section 2.6, Children's Susceptibility, and Section 5.6, Exposures of Children.

Minimal Risk Levels for 3,3'-Dichlorobenzidine.

Inhalation MRLs.

No acute-duration inhalation MRL was calculated for 3,3'-dichlorobenzidine due to the inadequate data. The information provided in the single relevant study in animals that is available (Gerarde and Gerarde 1974) is severely limited by lack of detailed reporting of the results. Included among the limitations are lack of information concerning exposure concentration and failure to use control groups. No intermediateduration inhalation MRL was calculated for 3,3'-dichlorobenzidine because no intermediate-duration studies in humans or animals were located. No chronic-duration inhalation MRL was calculated for 3,3'-dichlorobenzidine because the available human studies do not provide quantitative exposure information (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Myslak et al. 1991). No chronicduration inhalation studies in animals were located.

Oral MRLs.

No acute-duration oral MRL was calculated for 3,3'-dichlorobenzidine because the available studies did not identify appropriate NOAELs or LOAELs (Ashby and Mohammed 1988; Birner et al. 1990; Cihak and Vontorkova 1987; Ghosal and Iba 1990). No intermediate-duration oral MRL was calculated for 3,3'-dichlorobenzidine because the available studies did not identify relevant noncancer effects (Ito et al.1983; Osanai 1976; Pliss 1959, 1963). No chronic-duration oral MRL was calculated for 3,3'-dichlorobenzidine because there were no NOAELs identified below the lowest available serious LOAEL for convulsions and slight neuronal degeneration in dogs (Stula et al. 1978).

Death. No deaths were reported in humans from inhalation, oral, or dermal exposure to 3,3'-dichlorobenzidine. In animals, 3,3'-dichlorobenzidine caused no deaths in rats exposed by the inhalation route in concentrations as high as 23,700 mg/m^3 for 2 hours per day for 7 days (Gerarde and Gerarde 1974). In

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addition, the estimated acute oral LD_{50} for rats (7,070 mg/kg for the free base and 3,820 mg/kg for thedihydrochloride salt) and the minimum dermal lethal dose for male and female New Zealand albino rabbits (>8,000 mg/kg) for 3,3'-dichlorobenzidine suggested that the lethal toxicity of 3,3'-dichlorobenzidine is minimal (Gerarde and Gerarde 1974). Consequently, it is unlikely that death will occur in humans exposed to 3.3'-dichlorobenzidine at the levels at which it occurs at hazardous waste sites.

Systemic Effects. Dermatitis appears to be the only effect of 3,3'-dichlorobenzidine (free base) exposure for which evidence exists in humans (Gerarde and Gerarde 1974). Gastrointestinal upset and upper respiratory tract infections have also been reported by workers, but the role of 3,3'-dichlorobenzidine was uncertain. 3,3'-Dichlorobenzidine has not been found to cause these effects in experimental animals.

Respiratory Effects. Upper respiratory infection and sore throat were among several principal reasons for frequent visits to a company's medical clinic by workers handling 3,3'-dichlorobenzidine dihydrochloride (dihydro salt of 3,3'-dichlorobenzidine) (Gerarde and Gerarde 1974). However, data from animal studies are equivocal regarding the etiology of these symptoms (Gerarde and Gerarde 1974). While it is possible that these symptoms were due to exposure to 3,3'-dichlorobenzidine hydrochloride, the irritant effects of HCl from the compound in combination with particulate toxicity could have been responsible for the observed effects in these studies. Therefore, it is not likely that respiratory ailments will occur in humans exposed to 3,3'-dichlorobenzidine at hazardous waste sites.

Cardiovascular Effects. Reports of cardiovascular effects in humans or animals after exposure to 3,3'-dichlorobenzidine by any route were not found in any of the existing epidemiological and animal studies, suggesting that the cardiovascular system is not a target of 3,3'-dichlorobenzidine toxicity. It is unlikely that cardiovascular effects will occur in humans exposed to 3,3'-dichlorobenzidine at levels found at hazardous waste sites.

Gastrointestinal Effects. Gastrointestinal upset was one of the symptoms reported by employees who worked with 3,3'-dichlorobenzidine dihydrochloride (dihydro salt of 3,3'-dichlorobenzidine) (Gerarde and Gerarde 1974). However, there is no conclusive evidence that 3,3'- dichlorobenzidine caused these gastrointestinal upsets since there was exposure to other chemicals as well. In addition, 3,3'-dichlorobenzidine has not been found to cause any of these effects in experimental animals. Therefore, it is unlikely

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that exposure to 3,3'-dichlorobenzidine at hazardous waste sites will cause gastrointestinal effects in humans.

Hematological Effects. No studies were located regarding hematological effects in humans after inhalation, oral, or dermal exposure to 3,3'-dichlorobenzidine. Although hematological effects may not be sensitive indicators for 3,3'-dichlorobenzidine toxicity, hemoglobin adducts were observed in animal studies following single oral exposures to 127 or 253 mg/kg 3,3'-dichlorobenzidine (Bimer et al. 1990) and repeated exposures to 0.3 mg/kg/day for up to 4 weeks (Joppich-Kuhn et al. 1997). Birner et al. (1990) suggested that metabolically formed nitroso derivatives and the formation of a sulfinic acid amide with cysteine residues in hemoglobin may be the mechanism of adduct formation. No hematological abnormalities were found in dogs exposed to 10.4 mg/kg/day 3,3'-dichlorobenzidine for 7 years (Stula et al.1978). Therefore, it is unlikely that blood abnormalities will occur in humans exposed to 3,3'-dichlorobenzidine at levels found at hazardous waste sites.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans or animals after exposure to 3,3'-dichlorobenzidine by any route. However, since this effect was not reported in any of the existing epidemiological and animal studies, it is unlikely that musculoskeletal effects will occur in humans exposed to 3,3'-dichlorobenzidine at levels found at hazardous waste sites.

Hepatic Effects. No studies were located regarding hepatic effects in humans after exposure to 3,3'-dichlorobenzidine. Information from animal studies on the liver effects of exposure to 3,3'-dichlorobenzidine suggests that exposure to sufficiently high levels of thecompound could cause liver injury as indicated by modest elevation in serum transaminase activity, fatty liver (Stula et al. 1978), decrease in hepatic vitamin E, and lipid peroxidation (Iba 1987a; Iba and Lang 1988; Iba and Thomas 1988). Some of these effects may contribute to the liver tumors induced. However, it is not known whether these liver injuries will occur in humans exposed to 3,3'-dichlorobenzidine at levels at which it occurs at hazardous waste sites since these effects were not reported in any worker studies in which exposures are significantly higher.

Renal Effects. No studies were located regarding renal effects in humans after exposure to 3,3'-dichlorobenzidine by any route. No effects to the kidneys or urinary parameters monitored were observed in dogs exposed to 10.4 mg/kg/day for up to 7 years (Stula et al. 1978). Based on these data, it is unlikely that

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kidney effects will occur in humans exposed to 3,3'-dichlorobenzidine at levels found at hazardous waste sites.

Endocrine Effects. No studies were located regarding endocrine effects in humans or animals after exposure to 3,3'-dichlorobenzidine by any route. However, since this effect was not reported in any of the existing epidemiological and animal studies, it is unlikely that endocrine effects will occur in humans exposed to 3,3'-dichlorobenzidine at levels found at hazardous waste sites.

Dermal Effects. Dermatitis was cited as the only verified health problem encountered by workers in contact with the free base of 3,3'-dichlorobenzidine in a dichlorobenzidine manufacturing plant (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.

Ocular Effects. No studies were located regarding ocular effects in humans after exposure to 3,3'-dichlorobenzidine by any route. No adverse effects on the eye were noted when dichlorobenzidine (isomer unspecified, free base) was directly placed in the conjunctival sac of the eye of rabbits (Gerarde and Gerarde 1974). However, 0.1 mL 3,3'-dichlorobenzidine dihydrochloride (dihydro salt of 3,3'-dichlorobenzidine) 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). Apparently, the irritant effects of hydrochloric acid from the salt-compound contributed to the observed effects. Based on these data, it is not probable that adverse effects to the eye will occur in humans exposed to 3,3'-dichlorobenzidine at levels at which it occurs at hazardous waste sites.

Body Weight Effects. No studies were located regarding body weight effects in humans after exposure to 3,3'-dichlorobenzidine by any route. No significant difference in body weight was observed in dogs exposed to 10.4 mg/kg/day for up to 7 years (Stula et al. 1978). Based on these data, it is unlikely that body weight effects will occur in humans exposed to 3,3'-dichlorobenzidine at levels found at hazardous waste sites.

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Metabolic Effects. No studies were located regarding metabolic effects in humans or animals after exposure to 3,3'-dichlorobenzidine by any route. However, since this effect was not reported in any of the existing epidemiological and animal studies, it is unlikely that metabolic effects will occur in humans exposed to 3,3'-dichlorobenzidine at levels found at hazardous waste sites.

Immunological and Lymphoreticular Effects. No studies were located regarding immunological and/or lymphoreticular effects in humans or animals following exposure to 3,3'-dichlorobenzidine by any route of exposure. The immune system does not appear to be a sensitive target of 3,3'-dichlorobenzidine toxicity. Consequently, immune system disruptions are not expected in humans exposed to 3,3'-dichlorobenzidine at the levels at which it occurs at hazard waste sites.

Neurological Effects. Workers exposed to 3,3'-dichlorobenzidine and possibly to other chemicals in a chemical manufacturing plant reported headache and dizziness at the company clinic (Gerarde and Gerarde 1974). No further information indicated neurological effects in humans following exposure to 3,3'-dichlorobenzidine. In animal studies, 1 of 6 dogs exhibited convulsions after 21, 28, and 42 months of oral treatment with 10.4 mg/kg/day 3,3'-dichlorobenzidine for 3.5 years. A necropsy of the dog at 42 months revealed slight neuronal degeneration at unspecified sites in the brain and/or spinal cord (Stula et al. 1978). In view of the fact that only one dog developed the lesion, direct causality cannot be inferred. In addition, based on its chemical structure, 3,3'-dichlorobenzidine does not appear to be a neurotoxicant. The information available suggests that at the levels found in the environment, 3,3'-dichlorobenzidine is unlikely to constitute a neurological hazard for humans.

Reproductive Effects. No studies were located regarding reproductive effects in humans or animals following exposure to 3,3'-dichlorobenzidine by any route of exposure. Consequently, reproductive system disruptions are not expected in humans exposed to 3,3'-dichlorobenzidine at the levels at which it occurs at hazard waste sites.

Developmental Effects. No studies were located regarding developmental effects of 3,3'-dichlorobenzidine in humans following brief or long-term exposure by any route. Abnormal growth was observed in kidneys explanted from fetuses of pregnant mice treated subcutaneously daily during the last week of pregnancy at an average daily dose of approximately 421 mg/kg (Shabad et al. 1972). Similarly, in subcutaneous-injection studies in BALB/C mice, hyperplastic foci and hyperchromic glomeruli were

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observed in kidneys of offspring of dams administered 2 mg 3,3'-dichlorobenzidine (about 93.5 mg/kg) 4 or 5 times throughout gestation (Golub 1970). In a study of similar design, by the same group of investigators, subcutaneous injection of 3,3'-dichlorobenzidine during pregnancy to mice resulted in the induction of tumors in the progeny (Golub et al. 1975). Because the pups were nursed by the dams, it is unknown whether these effects may have been caused by transplacental transfer of the active principle, through nursing, or both. The significance of these findings to human health is unclear, particularly because of the irrelevant route of exposure and the high doses used.

Genotoxic Effects. Studies in several test systems show 3,3'-dichlorobenzidine to be genotoxic *in vivo* and *in vitro* (see Tables 2-3 and 2-4). It has been suggested that genotoxicity of 3,3'-dichlorobenzidine mediates the carcinogenicity of the compound (Imaoka et al. 1997; Ghosal and Iba 1990).

In vivo, micronuclei were induced in polychromatic erythrocytes of the liver of fetal mice exposed transplacentally to the compound, and in liver cells of adult male mice treated orally with the compound 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. However, whether this differential effect extends to carcinogenic effects is unclear. Positive chromatid exchange findings in an *in vitro* test system provide supportive evidence for 3,3'-dichlorobenzidine-induced cytogenetic changes. In a study using type I, II, and III Bloom Syndrome (BS) B-lymphoblastoid cell lines, 3,3'-dichlorobenzidine induced sister chromatid exchanges (SCEs) in all three types (Shiraishi 1986). However, the induction of SCE was variable among the three types. Exposure of BS type II and type III cells to 3,3'-dichlorobenzidine ($1x10^{-8}$ to $1.3x10^{-3}$ M) caused an increase in SCEs (120-140/cell) over baseline levels (70/cell) at the highest concentration ($1.3x10^{-3}$ M). BS type II cells required metabolic activation, while BS type III cells were sensitive with and without activation. The frequency of SCEs in BS type I cells was lower than in II and III.

The genotoxic effect of 3,3'-dichlorobenzidine is further supported by positive responses in bacterial assays employing *Sulmonella* tester strains TA1538 and TA98 in the absence of liver activating systems (Garner et al. 1975; Iba 1987a; Iba and Thomas 1988; Lazear et al. 1979; Savard and Josephy 1986). In another study, 3,3'-dichlorobenzidine exhibited both direct and hydrogen peroxide-dependent mutagenicity in S. *thyphimurium* strain TA98, but not Tal00 or TA102, leading the authors to suggest that enzymes perhaps

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

Species (test system)	End point	Results	Reference
Mammalian cells			
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
DNA Binding			
Mouse (male)	Binding to DNA	+	Ghosal and Iba 1990
Rat (male)	Binding to DNA	+	Ghosal and Iba 1990
Rat (male)	Binding to DNA	+	Bratcher and Sikka 1982

+ = Positive result; − = Negative result

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

Species (test system)	End point	Activation system	Result		Reference
			With activation	Without activation	
Prokaryotic organisms:					
Salmonella typhimurium TA98	Gene mutation	Mouse liver S-9	+	+	Lazear et al. 1979
S. typhimurium TA98	Gene mutation	Hamster liver S-9	+	+	Savard and Josephy 1986
S. typhimurium TA98	Gene mutation	Rat liver S-9	+	ND	Vithayathil et al. 1983
S. typhimurium TA100	Gene mutation	Mouse liver S-9	−	−	Lazear et al. 1979
S. typhimurium NM2009	DNA damage	Mouse kidney S-9	+	ND	Imaoka et al. 1997
S. typhimurium NM2009	DNA damage	Mouse bladder S-9	+	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
Eukaryotic organisms					
B-lymphoblastoid cell line II	Sister chromatid exchange	Rat liver S-9	+	−	Shiraishi 1986
B-lymphoblastoid cell line III	Sister chromatid exchange	Rat liver S-9	+	+	Shiraishi 1986

ND = no data; − = Negative results; + = Positive results

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endogenous to the tester strain TA98 may play a role in the activation of 3,3'-dichlorobenzidine (Lang and Iba 1987). A mixture containing Arochlor-induced rat liver homogenate and 10 μg 3,3'-dichlorobenzidine was positive for reverse mutation in *S. typhimurium* strain TA98 (histidine revertants) (Vithayathil et al. 1983). A recent study reported DNA damage in *S. typhimurium* NM2009 after incubation with 3,3'-dichlorobenzidine activated by mouse kidney or bladder rnicrosomes or rat liver microsomes (Imaoka et al. 1997).

3,3'-Dichlorobenzidine is an effective inducer of its own activation (Iba 1987a). The enhancing effect of 3,3'-dichlorobenzidine pretreatment on the *in vitro* liver activation of the chemical to mutagens has been associated with the induction of cytochrome P-450d (CYPlA2) (Iba and Thomas 1988). This action may result in the compound enhancing its own genotoxicity and carcinogenicity. 3,3'-Dichlorobenzidine was also shown to be a potent inducer of hepatic microsomal enzymic activities mediated by cytochrome-P-448 (CYPlA2) and P-450 in other animal studies (Iba and Sikka 1983; Iba and Thomas 1988). In another study to evaluate the P-450 induction pattern of 3,3'-dichlorobenzidine, intraperitoneal administration of 20-120 mg/kg 3,3'-dichlorobenzidine to male Sprague-Dawley rats induced P-450 isozymes in a pattern similar to 3-methylcholanthrene (i.e., induced P-450c) (CYP2Bl), and P-450d (CYPlA2) but mainly P-450c (CYP2Bl). 3,3'-Dichlorobenzidine activation was unaffected by monoclonal antibodies to P-450b (CYPlAl) or P-450c (CYP2Bl) but was inhibited by 69% by polyclonal antibodies to P-450d (CYPlA2). 3,3'-Dichlorobenzidine activation was also inhibited by 46% by antibody specific to NADPH-cytochrome P-450 reductase. Based on these results, it was concluded that P-450d (CYPlA2) is mainly responsible for the activation of 3,3'-dichlorobenzidine to mutagens in the Ames test by rat hepatic microsomes (Iba et al. 1983).

Results of *in vivo* tests show that 3,3'-dichlorobenzidine induced dose-dependent unscheduled DNA synthesis in the liver of male rats treated orally (Ashby and Mohammed 1988). *In vitro* evidence for the genotoxicity of 3,3'-dichlorobenzidine includes the induction of UDS in HeLa cells at a concentration range of $10m^{-7}$ to $10^{-4}M$ (Martin et al. 1978), and transformation of high passage rat embryo cells infected with the Rauscher leukemia virus (Freeman et al. 1973). In the latter system, an effect was observed at $2x10^{-7}$ M 3,3'-dichlorobenzidine, but not at $4x10^{-8}$ M. Also, 3,3'-dichlorobenzidine transformed BHK21 cells (hamster kidney cells) *in vitro* in the presence of metabolic activation (Styles 1978). The UDS assay is used to measure the repair that follows DNA damage. However, the relevance of UDS to human health is not known. While results were positive in two *in vivo* assay systems, sufficient data are not available from

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more predictive indicator assays to adequately characterize the genotoxic potential for 3,3'-dichlorobenzidine in humans.

3,3'-Dichlorobenzidine formed adducts with calf thymus DNA when incubated with rat liver S9 (Bratcher and Sikka 1982), or horseradish peroxidase (Tsuruta et al. 1985) *in vitro*. 3,3'-Dichlorobenzidine was also shown to bind extensively to tissue DNA in rats and mice. 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). Results from *in vitro* studies in rats and mice indicated that 3,3'-dichlorobenzidine formed tissue DNA-binding derivatives of 3,3'-dichlorobenzidine (Ghosal and Iba 1990). However, the relevance of DNA adduct formation to the genotoxicity and carcinogenicity of the compound and to human health is not yet established. Therefore, the genotoxicity consequences of 3,3'-dichlorobenzidine in humans remain uncertain.

Cancer. Due, in part, to structure-activity considerations, epidemiological studies of potential cancer effects of occupational exposure to 3,3'-dichlorobenzidine have focused upon bladder tumors since benzidine is a known bladder carcinogen. One study found an excess incidence of bladder tumors among German painters who were exposed to various dyes and pigments derived from benzidine, 3,3'-dichlorobenzidine, 3,3-dimethylbenzidine (*o*-tolidine), 3,3-dimethoxybenzidine (*o*-dianisidine), and 2-naphthylamine (Myslak et al. 1991). Because of the potential exposure of the painters to multiple chemicals (including some known bladder carcinogens), the role of 3,3'-dichlorobenzidine in the increased incidence of bladder tumors, if any, is unknown. A more recent study found a significant increase in the incidence of bladder cancers among a group of about 700 employees employed at a Connecticut chemical plant (Ouellet-Hellstrom and Rench 1996). In this case there was no exposure to benzidine, but the workers were also exposed to several arylamines other than 3,3'-dichlorobenzidine, therefore risks from specific chemical exposures could not be evaluated.

No other epidemiological studies have found bladder tumors or excess tumors at other sites (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975). Cancer effects have not been satisfactorily investigated in these studies of occupationally exposed workers. These studies were conducted with workers who were exposed to 3,3'-dichlorobenzidine for less than 20 years. Since the latency period for chemically induced bladder cancer in humans ranges from 5 to 50 years (Badalament 1998), the induction period for

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3,3'-dichlorobenzidine-induced tumors may not have elapsed for some individuals. Also, the number of workers examined in these studies was relatively small, thus limiting the statistical power to detect a significant increase in bladder cancer mortality (incidence).

Some have speculated that 3,3'-dichlorobenzidine may have contributed to the incidence of bladder cancer attributed to benzidine in dye industry workers who handled both benzidine and 3,3'-dichlorobenzidine (Gadian 1975; IARC 1982a). No bladder tumors were observed in a group of workers who handled only 3,3'-dichlorobenzidine; in the same plant, bladder tumors were found among workers who handled both benzidine and 3,3'-dichlorobenzidine. The investigator reported a total exposure time of 68,505 hours for the study population, equivalent to nearly 140 full-time working years (Gadian 1975). Cytodiagnostic tests produced no indication of tumors of the bladder in an epidemiological study of 259 workers who had been exposed for a total of less than 16 years to 3,3'-dichlorobenzidine (MacIntyre 1975). In a retrospective epidemiological study, no bladder tumors were observed in a cohort of 207 workers, most of whom had been exposed for up to 15 years (Gerarde and Gerarde 1974). However, in this study there was no evidence that any valid system of medical surveillance of workers ever existed during the years that 3,3'-dichlorobenzidine was used at the plant (Gerarde and Gerarde 1974). A number of other inadequacies noted by reviewers of the study severely limit the study's usefulness.

In animal studies, 3,3'-dichlorobenzidine has been found to cause neoplasia in a variety of target organs in several species. The compound produces hepatocellular carcinomas and urinary bladder carcinomas in dogs and hamsters (Sellakumar et al. 1969; Stula et al. 1978). Liver cell tumors were demonstrated in mice exposed to 3,3'-dichlorobenzidine in the diet (Osanai 1976; Pliss 1959). In rats, mammary gland tumors, Zymbal gland tumors, urinary bladder tumors, and leukemias were attributable to 3,3'-dichlorobenzidine exposure (Pliss 1959, 1963; Stula et al. 1975). One cancer study of dogs which evaluated one sex and used one dose level (precluding dose-response evaluation) shows a sufficient number of animals survived to develop tumors (Stula et al. 1978). The results of a study in rats suggested that 3,3'-dichlorobenzidine may have a synergistic effect on the bladder carcinogenicity of other chemicals (Ito et al. 1983).

Because of the increased use of closed systems and protective clothing, dermal absorption of 3,3'-dichlorobenzidine probably represents a relatively minor route of exposure (EPA 1980b). However, there is experimental evidence that under certain environmental conditions favoring moist skin conditions, such as high relative humidity and high air temperature, dermal absorption of 3,3'-dichlorobenzidine by

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humans may be enhanced (Meigs et al. 1954). Studies have not been located which investigate the carcinogenic potential of 3,3'-dichlorobenzidine following dermal exposure in laboratory animals.

Further evidence of the carcinogenic potential of 3,3'-dichlorobenzidine is provided by studies where 3,3'-dichlorobenzidine was administered subcutaneously. Following subcutaneous administration in rats for 10 to 13 months, the compound was found to cause tumors of the skin, sebaceous and mammary glands, and urinary bladder (Pliss 1963). These sites were in addition to tumors of the hematopoietic tissues and Zymbal gland which were observed following oral exposure (Pliss 1959). Pliss (1963) further indicated that oral exposure to 3,3'-dichlorobenzidine resulted in a higher incidence of tumors in rats than after subcutaneous injection of the compound. Pliss (1963) also noted that the introduction of chlorine into the benzidine molecule resulted in an increased carcinogenic response in the skin and the urinary bladder. Local subcutaneous sarcomas and liver tumors were observed in 13/28 strain D mice following subcutaneous administration of 3,3'-dichlorobenzidine for 11 months (Pliss 1959).

In subcutaneous injection studies, induction of tumors in the progeny of BALB/c mice administered 2 mg 3,3'-dichlorobenzidine (about 93.5 mg/kg) 4 or 5 times during the last week of pregnancy suggest that the chemical may be a transplacental carcinogen (Golub et al. 1975). There was an increased incidence of lymphatic leukemias (7 of 24, 29%), lung adenomas (5 of 24, 20%), and adenocarcinomas of the mammary gland (4 of 11 female offspring, 36%) in the treated group. Lung tumors (3 of 30 offspring, 10%) and mammary gland tumors (3 of 19 female offspring, 16%) were observed in untreated controls (Golub et al. 1975). It should be noted that since the offspring were nursed by the treated dams, transfer of 3,3'-dichlorobenzidine to the offspring through maternal milk may have also occurred.

3,3'-Dichlorobenzidine is an effective inducer of its own metabolic activation (Iba 1987a). The enhancement of 3,3'-dichlorobenzidine mutagenesis has been associated with the induction of cytochrome P-450d (Iba and Thomas 1988), and may result in the elevation of its carcinogenicity. In other animal studies, 3,3'-dichlorobenzidine was also shown to be a potent inducer of hepatic microsomal enzymic activities mediated by cytochrome-P-448 and P-450 (Iba and Sikka 1983; Iba and Thomas 1988). Consequently, it has been suggested that the hepatocarcinogenicity of 3,3'-dichlorobenzidine may be due, at least in part, to the induction of hepatic cytochrome P-488 and DNA-adduction.

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While concordance between tumor sites in experimental animals and humans cannot be assumed, the occurrence of tumors in multiple organs in several species of experimental animals should be regarded as evidence for the potential carcinogenicity of 3,3'-dichlorobenzidine to humans.

The Environmental Protection Agency (EPA) has determined that 3,3'-dichlorobenzidine is a probable human carcinogen. The U.S. Department of Health and Human Services (DHHS) has determined that 3,3'-dichlorobenzidine and its dihydrochloride salt may reasonably be expected to be carcinogens. IARC (1987) has determined that 3,3'-dichlorobenzidine is possibly carcinogenic to humans.

2.6 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate due to maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 5.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both pre-natal and post-natal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of

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their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns and at various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults and sometimes unique enzymes may exist at particular developmental stages (Komori 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in the newborn who has a low glomerular filtration rate and has not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility while others may decrease susceptibility to the same chemical. For example, the fact that infants breathe more air per kilogram of body weight than adults may be somewhat counterbalanced by their alveoli being less developed, so there is a disproportionately smaller surface area for absorption (NRC 1993).

No studies were located that specifically addressed the health effects of exposure to 3,3'-dichlorobenzidine in children. Limited data in adults are mostly derived from occupational studies with limitations including lack of precise exposure data and presence of other compounds. As a result, 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. Thus far, largely because of study limitations, there is no conclusive evidence that exposure to 3,3'-dichlorobenzidine increases the risk of bladder cancer in humans (Gadian 1975; Gerarde and Gerarde 1974; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996).

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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 1970; 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. Although there have been no direct measurements to determine whether 3,3'-dichlorobenzidine can cross the placenta, there is some indirect evidence that it or its metabolites do. The evidence is based on the results of a study in which oral administration of 3,3'-dichlorobenzidine to pregnant mice resulted in the induction of micronuclei in the liver of fetuses (Cihak and Vontorvoka 1987). The results of another study in which subcutaneous administration of 3,3'-dichlorobenzidine to pregnant mice induced abnormal growth of the kidneys explanted from the fetuses also suggest that 3,3'-dichlorobenzidine or a metabolite can cross the placenta (Shabad et al. 1972). There is no information 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 done by one of two families of N-acetyltransferases. One of these families, NAT2, is developmentally regulated (Leeder and Kearns 1997). Some enzyme activity can be detected in the fetus by the end of the first trimester. Almost all infants exhibit the slow acetylator phenotype between birth and 2 months of age. The adult phenotype distribution is reached by the age of 4-6 months, whereas adult activity is found by approximately l-3 years of age. Also, UDP-glucuronosyltransferase, responsible for the formation of glucuronide conjugates, seems to achieve adult activity by 618 months of age (Leeder and Keams 1997). These data suggest that metabolism of 3,3'-dichlorobenzidine by infants will differ from that in adults in extent, rate, or both.

There are no biomarkers of exposure or effect for 3,3'-dichlorobenzidine that have been validated in children or adults exposed as children. There are no biomarkers in adults that identify previous childhood exposure. No studies were located regarding interactions of 3,3'-dichlorobenzidine with other chemicals in children or adults. No studies were located that examined possible differential susceptibility between young and older organisms.

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No information was located regarding 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.

2.7 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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 (NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 3,3'-dichlorobenzidine are discussed in Section 2.7.1.

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

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potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by
3,3'-dichlorobenzidine are discussed in Section 2.7.2.

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 2.9, Populations That
Are Unusually Susceptible.

2.7.1 Biomarkers Used to Identify or Quantify Exposure to 3,3'-Dichlorobenzidine

A test system that involves extracting dichlorobenzidine or its metabolite
(monoacetyldichlorobenzidine) from urine and reacting it with Chloramine-T has been developed
to screen for dichlorobenzidine exposure in workers (Hatfield et al. 1982). An amperometric
method has been developed for the detection of 3,3'-dichlorobenzidine in the urine as a
quantitative assay for the biological monitoring of people occupationally exposed to this
substance or a metabolic precursor such as certain pigments. This method is based on the
possibility of two electron oxidation at carbon electrodes by aromatic diamines (Trippel-
Schulte et al. 1986).

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. Although these
methods have not yet been validated in an occupationally exposed population, they appear
potentially suitable for use as a biomarker of human exposure to 3,3'-dichlorobenzidine.

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2.7.2 Biomarkers Used to Characterize Effects Caused by 3,3'-Dichlorobenzidine

For more information on biomarkers for renal and hepatic effects of chemicals, see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990). For information on biomarkers for neurological effects, see OTA (1990).

Currently no disease states in humans are clearly associated with exposure to 3,3'-dichlorobenzidine. There is evidence that 3,3'-dichlorobenzidine is carcinogenic in animals (Golub et al. 1975; Osanai 1976; Pliss 1959, 1963; Sellakumar et al. 1969; Stula et al. 1975, 1978) and that it is genotoxic in test systems (Ashby and Mohammed 1988; Cihak and Vontorkova 1987; Ghosal and Iba 1990; Shiraishi 1986). Hemoglobin adducts have been isolated from the blood of 3,3'-dichlorobenzidine-treated animals (Birner et al. 1990; Joppich-Kuhn et al. 1997), although further studies are needed to determine the associations between blood levels of these adducts and specific adverse effects.

2.8 INTERACTIONS WITH OTHER CHEMICALS

In contrast to its effects on other mutagens and carcinogens, di-tert,-butylated hydroxytoluene (BHT), 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-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).

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)-2-thiazolyllformamide) 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 authors suggested that 3,3'-dichlorobenzidine had a synergistic effect on the carcinogenicity of BBN. In rats sequentially administered BBN (0.01%),

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FANFT (0.15%) 2-AAF (0.025%), and 3,3'-dichlorobenzidine (0.03%) for 4 weeks, the incidence ofbladder 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).

2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population is defined as one which will exhibit a different or enhanced response to a chemical compared to most persons exposed to the same level of exposure. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). For this chemical, these parameters may result in reduced detoxification or excretion of 3,3'-dichlorobenzidine, or compromised function of target organs affected by 3,3'-dichlorobenzidine. Populations who are at greater risk due to their unusually high exposure to 3,3'-dichlorobenzidine are discussed in Section 5.6, Populations With Potentially High Exposure.

No information was located that identified any human population that is exceptionally susceptible to the toxicity of 3,3'-dichlorobenzidine. See Section 2.6, Children's Susceptibility, for a discussion of that topic.

2.10 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 3,3'-dichlorobenzidine. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to 3,3'-dichlorobenzidine. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.10.1 Reducing Peak Absorption Following Exposure

The only information in the literature regarding reducing the absorption of 3,3'-dichlorobenzidine was found in a Fact Sheet published by the State of New Jersey (State of New Jersey 1997). The recommendations source indicate that following eye contact, eyes should immediately be flushed with large amounts of water for at least 15 minutes, occasionally lifting upper and lower lids. It is also recommended that after skin contact contaminated clothing should be quickly removed and contaminated skin should be

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immediately washed with large amounts of soap and water. A person exposed to
3,3'-dichlorobenzidine in the air should be removed from the source of exposure promptly.

Other information specific for 3,3'-dichlorobenzidine, aimed at minimizing exposure, was found
in the HSDB database (HSDB 1997). This information indicates that full body protective clothing
and gloves should be used by those employed in handling operations. Full face supplied air
respirators of continuous flow or pressure demand should also be used. In addition, employees
working with 3,3'-dichlorobenzidine (or its salts) within an isolated system, such as "glove box,"
should wash their hands and arms upon completion of the assigned task and before engaging in
other activities not associated with the isolated system.

2.10.2 Reducing Body Burden

There are no established methods for reducing the body burden of 3,3'-dichlorobenzidine.

2.10.3 Interfering with the Mechanism of Action for Toxic Effects

There are no known methods for interfering with the toxic effects of 3,3'-dichlorobenzidine.

2.11 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 the National Toxicology
Program (NTP), is required to assure the initiation of a program of research designed to determine
the health effects (and techniques for developing methods to determine such health effects) of
3,3'-dichlorobenzidine.

The following categories of possible data needs have been identified by a joint team of scientists
from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if
met would reduce the uncertainties of human health assessment. This definition should not be
interpreted to mean that

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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.

2.11 .l Existing Information on Health Effects of 3,3'-Dichlorobenzidine

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 3,3'-dichlorobenzidine are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of 3,3'-dichlorobenzidine. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Zdentifiing 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.

Essentially no studies of human exposure to 3,3'-dichlorobenzidine were located by specific routes, except for occupational data on direct dermal effects following dermal exposure and a recent carcinogenicity study in which skin contact with 3,3'-dichlorobenzidine and other arylamines was found to be the most important exposure route (see Figure 2-3). Although there are studies of workers in the United States exposed to 3,3'-dichlorobenzidine, these reports are limited by the fact that exposure often involved other compounds, and both the route and extent of exposure are largely unknown. Dermal effects have also been investigated in experimental animals as well as ocular irritant properties of 3,3'-dichlorobenzidine exposure. There is no evidence to suggest that the non-ocular systemic toxicological effects of 3,3'-dichlorobenzidine may be route- or species-specific.

Additional information on health effects following dermal exposure is sparse. The majority of animal studies of 3,3'-dichlorobenzidine have focused on carcinogenic effects following oral exposure, whereas data on noncarcinogenic effects are limited.

Figure 2-3. Existing Information on Health Effects of 3,3'-Dichlorobenzidine

Human

Animal

●   Existing Studies

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2.11.2 Identification of Data Needs

Acute-Duration Exposure. One study in humans showed that the compound may cause respiratory effects when inhaled and that application of 3,3'-dichlorobenzidine base causes skin irritation (Gerarde and Gerarde 1974). Thus, this 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, corneal opacity) in rabbits following conjunctival application. However, the relevance of these findings for the general population is unknown since conjunctival application is not a typical route of exposure, and exposure by the inhalation route 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 threat 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 MRLs.

Intermediate-Duration Exposure. No intermediate-duration studies in humans were located. Intermediate-duration oral studies have been performed in rats without adverse systemic effects, but these studies used only one dose level (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 which could prove more predictive when assessing potential adverse effects in humans following intermediate-duration exposure. No oral intermediate MRL was derived because the available studies did not identify relevant noncancer effects.

Chronic-Duration Exposure and Cancer. No studies were located that examined noncancer end points in humans following chronic exposure to 3,3'-dichlorobenzidine. 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 toxicological parameters measured were limited. 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,

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and oral studies involving low-dose exposure in animals might provide dose-response data on potential systemic effects of exposure in 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 workers exposed to it (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996). 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; Sellakumar et al. 1969; 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)

Genotoxicity. Available studies in animals and in bacterial systems show that 3,3'-dichlorobenzidine does alter genetic material (Ashby and Mohammed 1988; Bratcher and Sikka 1982; Cihak and Vontorkova 1987; Garner et al. 1975; Iba 1987a; Iba and Thomas 1988; Imaoka et al. 1997; Lang and Iba 1987; Lazear et al. 1979; Savard and Josephy 1986; Shiraishi 1986; Styles 1978). Studies involving more predictive indicator test systems may allow a better assessment of mutagenic potential.

Reproductive Toxicity. No studies were found regarding reproductive toxicity of 3,3'-dichlorobenzidine. Should data suggesting that reproductive organs are affected in a 90-day study become available, multigenerational reproductive studies in animals may be warranted.

Developmental Toxicity. No studies were found regarding developmental toxicity of 3,3'-dichlorobenzidine in humans. Animal studies have shown that 3,3'-dichlorobenzidine and/or 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 1972; Shabad et al. 1972) or induce tumors

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in the offspring (Golub et al. 1975). The effects of the compound on development following oral, inhalation, or dermal exposure have not been studied. Well conducted animal studies employing 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 nursing. 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.

Immunotoxicity. No studies were located assessing the potential effect on the immune system during 3,3'-dichlorobenzidine exposure. Studies that examine antibody levels and responses to bacterial infections after exposure to 3,3'-dichlorobenzidine would provide valuable information on the immune system. Also, evaluation of morbidity among individuals exposed to 3,3'-dichlorobenzidine in the workplace may provide important indirect evidence regarding their immune status.

Neurotoxicity. Based on its chemical structure, 3,3'-dichlorobenzidine does not appear to be neurotoxicant, but the nervous system has not been carefully evaluated after exposure to this chemical. Workers exposed to 3,3'-dichlorobenzidine (and to other chemicals as well) complained of headache and dizziness (Gerarde and Gerarde 1974). 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 this 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, and studies aimed exclusively to evaluate this end point seem unnecessary at this time. 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 end points 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.

Epidemiological 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. Workers exposed to 3,3'-dichlorobenzidine (and simultaneously to other chemicals) have complained of gastrointestinal upset, upper respiratory infection,

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sore throat, caustic burns, headache, dizziness, and dermatitis (Gerarde and Gerarde 1974). The only one of these effects that appeared to be associated with 3,3'-dichlorobenzidine exposure with reasonable certainty is dermatitis, which was attributed to a manufacturing process change that resulted in exposure to dichlorobenzidine-freebase (Gerarde and Gerarde 1974). Studies of occupationally exposed individuals are 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 beendetected in groundwater, near industries releasing 3,3'-dichlorobenzidine, or near hazardous waste sites could provide information on whether 3,3'-dichlorobenzidine exposure produces effects in humans. In the unlikely event that exposure of the general population (in the past or present) primarily to 3,3'-dichlorobenzidine is identified, individuals should be monitored for gastrointestinal, respiratory, dermal, and neurological 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.

Exposure. A test system that involves extracting dichlorobenzidine or its metabolite (monoacetyldichlorobenzidine) from urine and reacting it with Chloramine-T has been developed to screen for dichlorobenzidine exposure in workers (Hatfield et al. 1982). In addition, an amperometric method has been developed for the detection of 3,3'-dichlorobenzidine in the urine as a quantitative assay for the biological monitoring of occupationally exposed persons to this substance. This method is based on the two electron oxidation at carbon electrodes by aromatic diamines (Trippel-Schulte et al. 1986). Hemoglobin adducts have been detected in female Wistar rats orally administered single doses of 127 or 253 mg/kg 3,3'-dichlorobenzidine (Birner et al. 1990) and to repeated doses of 0.3 mg/kg/day (Joppich-Kuhn et al. 1997). Birner et al.(1990) suggested that metabolically formed nitroso derivatives can result in the formation of a sulfinic acid amide with cysteine residues in hemoglobin. Hydrolysis yielded mainly 3,3'-dichlorobenzidine; *N*-acetylated-3,3'-dichlorobenzidine was also detected. This method has not yet been validated in an occupationally exposed population. More research is needed to determine if this method is suitable for use

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as a biomarker of human exposure to 3,3'-dichlorobenzidine. Further studies to develop simpler, more sensitive biomarkers of exposure that are specific for 3,3'-dichlorobenzidine would be useful in monitoring exposure of people living near hazardous waste sites containing 3,3'-dichlorobenzidine.

Effect. 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). 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 in establishing the relevance of animal studies in predicting human health effects. Metabolic handling of 3,3'-dichlorobenzidine in humans needs to be better characterized before urinary levels of the compound or its metabolites can be used 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. Pharmacokinetics 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.

Methods for Reducing Toxic Effects. There are no disease states in humans that are associated with exposure to 3,3'-dichlorobenzidine. Therefore, studies that further characterize means of assessing human exposures (biomonitoring) along with identification of programs designed to minimize this exposure would be effective for mitigation of potential effects resulting from accidental exposure in occupational settings or exposure to humans living near hazardous waste sites where 3,3'-dichlorobenzidine might be stored.

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Children's Susceptibility. The information on health effects of 3,3'-dichlorobenzidine in humans is derived exclusively from studies of occupational exposure (Gadian 197.5; Gerarde and Gerarde 1974; MacIntyre 1975; Myslak et al. 1991; Quellet-Hellstron and Rench 1996). Because of study limitations such as simultaneous exposure to other chemicals, no target organ or system has been identified for 3,3'-dichlorobenzidine. In one occupational study it was reported that contact with the free base 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 unrealistic. 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 either, but given the unlikelihood of exposure to 3,3'-dichlorobenzidine by the general population, such studies do seem warranted at this time.

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 1970; 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 route of exposure. This also includes information on whether 3,3'-dichlorobenzidine (or metabolites) can cross the placenta and/or be transferred to offspring via breast milk. There are no data to evaluate whether harmacokinetics of 3,3'-dichlorobenzidine in children are different from adults. There are no PBPK models for 3,3'-dichlorobenzidine, but a need for such a model is not apparent at this time. There is no information to evaluate whether metabolism of 3,3'-dichlorobenzidine in children is different than in adults, but there are some theoretical reasons to suspect that it might be different.

Continued research into the development of sensitive and specific biomarkers of exposure and effect for 3,3'-dichlorobenzidine, and the validation of these biomarkers in occupationally exposed individuals would be valuable. Since at this point there are no validated biomarkers of exposure and effect in adults, it makes sense to focus efforts on occupationally exposed adults rather than children who are unlikely to be exposed. There are no data on interactions of 3,3'-dichlorobenzidine with other chemicals in children or adults. There are no pediatric-specific methods to reduce peak absorption for 3,3'-dichlorobenzidine following exposure, to reduce body burdens, or to interfere with 3,3'-dichlorobenzidine's mechanism of action, but it is reasonable to assume that exposure avoidance measures should be applied to children where needed.

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Child health data needs relating to exposure are discussed in Section 5.8.1, Data Needs: Exposures of Children.

2.11.3 Ongoing Studies

No ongoing studies were located for 3,3'-dichlorobenzidine (FEDRIP 1998).