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

3.1 TOXICOKINETICS

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

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

3.1.1 Absorption

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

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

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

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

3.1.2 Distribution

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

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

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

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

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

3.1.3 Metabolism

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

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

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

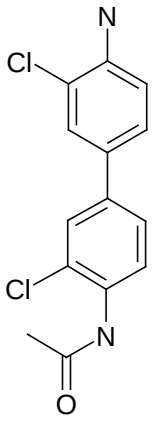
Studies in animals also indicate that 3,3'-dichlorobenzidine is extensively metabolized. Bile and urine of rats given single oral doses of [^{14}C]-3,3'-dichlorobenzidine (40 mg/kg/day) contained five metabolites of 3,3'-dichlorobenzidine in addition to the parent compound. However, none of the metabolites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

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

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

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

N-acetyl-3,3'-dichlorobenzidine



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

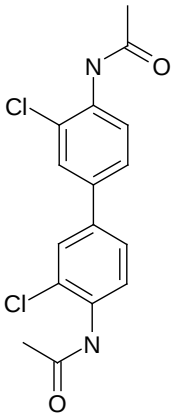


Figure 3-2. Potential Form of the Nitroso Intermediate

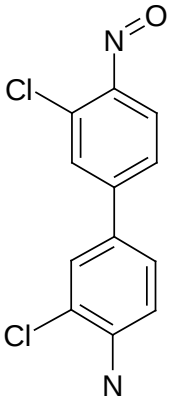
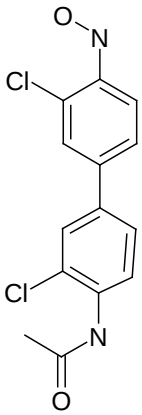
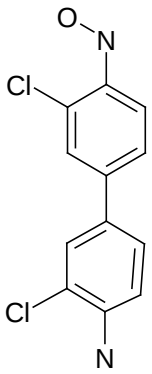


Figure 3-3. Hydroxy- Metabolites of 3,3'-Dichlorobenzidine

N-Hydroxy-N'-acetyl-dichlorobenzidine



N-Hydroxy-dichlorobenzidine



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

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

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

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

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

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

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

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

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

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

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

3.1.4 Excretion

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

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

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

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

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

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

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

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

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

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

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

3.1.6 Animal-to-Human Extrapolations

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

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

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

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

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

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

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

3.3.1 Biomarkers of Exposure

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

3.3.2 Biomarkers of Effect

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

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

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

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

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

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

3.4 INTERACTIONS WITH OTHER CHEMICALS

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

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