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HEALTH AND ENVIRONMENTAL EFFECTS DOCUMENT FOR 3,3'-DICHLOROBENZIDINE

Prepared for

OFFICE OF SOLID WASTE AND
EMERGENCY RESPONSE

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PREFACE

Health and Environmental Effects Documents (HEEDs) are prepared for the Office of Solid Waste and Emergency Response (OSWER). This document series is intended to support listings under the Resource Conservation and Recovery Act (RCRA) as well as to provide health-related limits and goals for emergency and remedial actions under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained for Agency Program Office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched for in this document and the dates searched are included in "Appendix: Literature Searched." Literature search material is current up to 8 months previous to the final draft date listed on the front cover. Final draft document dates (front cover) reflect the date the document is sent to the Program Officer (OSWER).

Several quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Reference doses (RfDs) for chronic and subchronic exposures for both the inhalation and oral exposures. The subchronic or partial lifetime RfD, is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs during a limited time interval i.e., for an interval that does not constitute a significant portion of the lifespan. This type of exposure estimate has not been extensively used, or rigorously defined as previous risk assessment efforts have focused primarily on lifetime exposure scenarios. Animal data used for subchronic estimates generally reflect exposure durations of 30-90 days. The general methodology for estimating subchronic RfDs is the same as traditionally employed for chronic estimates, except that subchronic data are utilized when available.

In the case of suspected carcinogens, RfDs are not estimated. Instead, a carcinogenic potency factor, or q_1^* (U.S. EPA, 1980) is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity, and acute mammalian toxicity). Chemical-specific RQs reflect the lowest of these six primary criteria. The methodology for chronic toxicity and cancer based RQs are defined in U.S. EPA, 1984 and 1986a, respectively.

EXECUTIVE SUMMARY

3,3'-Dichlorobenzidine (CAS number 91-94-1) is a gray to purple crystalline solid at room temperature (Hawley, 1981). It is available commercially in the form of its dihydrochloride salt (IARC, 1982). Commercial production involves alkaline reduction of o-chloronitrobenzene, followed by rearrangement with hydrochloric acid to form 3,3'-dichlorobenzidine dihydrochloride (IARC, 1982). Bofors Nobel, Inc. in Muskegon, MI, and The Upjohn Co. in North Haven, CT, are currently the only U.S. manufacturers of 3,3'-dichlorobenzidine dihydrochloride (SRI, 1987). 3,3'-Dichlorobenzidine dihydrochloride is imported into the United States; during 1983, 1.104 million pounds of 3,3'-dichlorobenzidine base and salts was imported through the principal U.S. customs districts (USITC, 1984). Essentially 100% of all 3,3'-dichlorobenzidine (in the form of its dihydrochloride) consumed in the United States is used as an intermediate for organic pigments (IARC, 1982; HSDB, 1987).

In the atmosphere, 3,3'-dichlorobenzidine is expected to exist primarily in the particulate form. 3,3'-Dichlorobenzidine, in both vapor and particulate form, is expected to undergo rapid photolysis in the atmosphere. Reaction of 3,3'-dichlorobenzidine vapor with photochemically generated hydroxyl radicals ($t_{1/2}$=10 hours) (Atkinson, 1987) may be a minor removal mechanism. In water, this compound would undergo rapid photodegradation ($t_{1/2}$=90 sec) in the surface layers of water (Banerjee et al., 1978). This compound photodegrades to monochlorobenzidine, benzidine and a number of brightly colored water-insoluble compounds (Banerjee et al., 1978). Beyond the reach of light penetration, this compound would rapidly adsorb to sediment and particulate matter where it is tightly bound. Adsorption is

expected to proceed initially by a rapid, but reversible, physical adsorption process followed by much slower irreversible covalent bonding (Appleton et al., 1978; Sikka et al., 1978). It has also been speculated that 3,3'-dichlorobenzidine may be oxidized by naturally occurring cations (e.g., Fe^{+3}) found in sediments (Callahan et al., 1979). Rapid uptake and bioaccumulation in aquatic organisms is also expected to occur (Appleton and Sikka, 1980; Freitag et al., 1985). Volatilization, microbial degradation and chemical hydrolysis are not expected to be important fate processes in water. In soil, 3,3'-dichlorobenzidine is expected to adsorb tightly to soil, and over time, irreversibly bind with humates in the soil (Boyd et al., 1984). It has also been speculated that oxidation by reaction with cationic constituents of soil (e.g., Fe^{+3}, Cu^{+3}) may also occur (Callahan et al., 1979; Demirjian et al., 1987). If exposed to sunlight on soil surfaces, 3,3'-dichlorobenzidine is expected to photodegrade rapidly. Volatilization and microbial degradation are not expected to be significant fate processes (Callahan et al., 1979).

Exposure to 3,3'-dichlorobenzidine is most likely to occur in occupational settings, particularly where this compound is manufactured or where 3,3'-dichlorobenzidine-based dyes are manufactured or used (HSDB, 1987). The general public may be exposed to low levels of 3,3'-dichlorobenzidine during use of paints, pigments or enamels derived from this compound. The most probable routes of exposure are inhalation of dusts or mists containing this compound and dermal contact (HSDB, 1987). 3,3'-Dichlorobenzidine has been detected in samples of surface water, sediment, fish and industrial effluent (U.S. EPA, 1981, 1987b; IARC, 1982; Hauser and Bromberg, 1982). This compound has not been detected in urban runoff samples collected as part of the U.S. EPA Nationwide Urban Runoff Program (Cole et al., 1984).

The U.S. EPA STORET Data Base indicates that the mean concentrations of 3,3'-dichlorobenzidine in whole water, sediment (wet and dry weight basis) and fish tissue samples collected throughout the United States are 46 µg/ℓ, 3222 µg/kg dry wt., 0.026 and 5.8 mg/kg wet wt., respectively (U.S. EPA, 1987b).

There are few data regarding the aquatic toxicity of 3,3'-dichlorobenzidine. A study by Appleton et al. (1978) indicated that in bluegills, Lepomis macrochirus, toxic levels of 3,3'-dichlorobenzidine accumulated before a 3,3'-dichlorobenzidine equilibrium was reached between water and fish. There were many mortalities when the whole body residues of fish exposed to ^{14}C-3,3'-dichlorobenzidine exceeded 150 ppm.

The absorption of 3,3'-dichlorobenzidine following administration of the compound by relevant routes (i.e., inhalation or oral exposure) has not been studied extensively. Hsu and Sikka (1982) reported that 3,3'-dichlorobenzidine is rapidly and extensively absorbed following oral administration of the compound to rats. Meigs et al. (1954) and Suskind (1983) reported that the skin is the most significant route of entry of 3,3'-dichlorobenzidine into the body in cases of occupational exposure. The half-life of disappearance of a topically applied 3,3'-dichlorobenzidine dose from the shaved backs of rats was determined to be 24.1 hours (Shah and Guthrie, 1983).

Following a single oral dose of radiolabled 3,3'-dichlorobenzidine to rats, the principal organs in which radioactivity was found were the liver, kidneys, lungs and spleens (Hsu and Sikka, 1982). Multiple oral 3,3'-dichlorobenzidine dosing led to tissue levels of radioactivity 3-4 times higher than the levels observed following a single oral dose, but a multiple dosing schedule did not result in substantial retention of radioactivity (Hsu and Sikka, 1982).

The extent of metabolism and the pathways of 3,3'-dichlorobenzidine metabolism are not clear. Ring hydroxylation products of 3,3'-dichlorobenzidine were not found in the urine of humans and dogs given an oral 3,3'-dichlorobenzidine dose (Troll, n.d.), and Shriner et al. (1978) suggested that chlorination of benzidine blocks ring hydroxylation reactions of 3,3'-dichlorobenzidine for both electronic and steric reasons. Hsu and Sikka (1982), however, provided evidence that 3,3'-dichlorobenzidine is metabolized extensively in rats.

Several possible metabolites of 3,3'-dichlorobenzidine, tentatively identified by chromotographic procedures, include mono-N-acetyl 3,3'-dichlorobenzidine in the urine of monkeys (Kellner et al., 1973) and benzidine and some possible glucuronide conjugates in the urine of rats (Aksamitnaia, 1959).

Elimination of both radiolabeled 3,3'-dichlorobenzidine and total radioactivity from the plasma of orally dosed rats was biphasic showing an initial rapid decline followed by a slower disappearance phase (Hsu and Sikka, 1982). Similarly, biphasic elimination of total radioactivity was observed in the principal organs of distribution (i.e., liver, lung and kidney and spleen) (Hsu and Sikka, 1982).

The bile appears to be a significant route of excretion for both 3,3'-dichlorobenzidine and its metabolites. Experiments with rats indicated that ~90% of the administered radioactivity is excreted in the urine and feces following an oral dose of 3,3'-dichlorobenzidine (Hsu and Sikka, 1982). Approximately 65% of the administered radioactivity was excreted in the feces and the major source of the radioactivity found in the feces originated from the bile. Hepatobiliary excretion of 3,3'-dichlorobenzidine and its metabolites also occurred in rhesus monkeys (Kellner et al., 1973).

The fecal route appears to be the most significant route of elimination of 3,3'-dichlorobenzidine and metabolites in humans (Troll, n.d.); 3,3'-dichlorobenzidine has also been reported to be present in the urine of occupationally-exposed individuals (Meigs et al., 1954; London and Boiano, 1986; Singal and Lee, 1985).

Pertinent data regarding the systemic toxicity of 3,3'-dichlorobenzidine following either subchronic or chronic inhalation exposure in humans and animals were not located in the available literature. Stula et al. (1978) indicated that beagle dogs exposed to 3,3'-dichlorobenzidine orally for periods up to 7.1 years showed signs of liver toxicity in the form of elevated SGPT activities. The oral LD_{50} of 3,3'-dichlorobenzidine in rats has been reported to be ~4 and 7 g/kg bw for dihydrochloride salt and 3,3-dichlorobenzidine, respectively (ACGIH, 1986).

Pertinent data regarding the carcinogenicity of 3,3'-dichlorobenzidine following inhalation exposure in humans and animals were not located in the available literature cited in Appendix A. Oral administration of 3,3'-dichlorobenzidine has been shown to produce a variety of tumors in rats (Pliss, 1959; Stula et al., 1975; Griswold et al., 1968), urinary bladder and liver tumors in dogs (Stula et al., 1978) and hamsters (Sellakumar et al., 1969), and hepatomas in mice (Osanai, 1976). Subcutaneous administration of 3,3'-dichlorobenzidine has also been demonstrated to produce tumors in rats (Pliss, 1959, 1963).

Both additive and synergistic tumorigenic effects were noted in rats following simultaneous or sequential administration of low levels of 3,3'-dichlorobenzidine along with low levels of other carcinogens (i.e., BBN alone or BBN, FANFT and 2-AAF sequentially (Ito et al., 1983). 3,3'-Dichlorobenzidine has also been demonstrated to function as a transplacental carcinogen in mice (Golub et al., 1975).

3,3'-Dichlorobenzidine has been suspected of being a human carcinogen because of its carcinogenic effects in animals and because it resembles benzidine, a known human bladder carcinogen. Evidence from three epidemiological studies (Gerarde and Gerarde, 1974; MacIntyre, 1975; Gadian, 1975), however, is inadequate to suggest that 3,3'-dichlorobenzidine is a bladder carcinogen in humans.

3,3'-Dichlorobenzidine has been demonstrated to be mutagenic towards Salmonella typhimurium in the Ames assay, both with and without metabolic activation. Metabolic activation (i.e., presence of liver S-9) has, however, been demonstrated to increase the mutagenicity of 3,3'-dichlorobenzidine from 3 to 50-fold (Garner, 1975; Lazear and Louie, 1977; DeFrance et al., 1986). 3,3'-Dichlorobenzidine has also been demonstrated to be active in in vitro assays measuring unscheduled DNA synthesis (Martin et al., 1978) and sister chromatid exchange (Shiraishi, 1986).

Pertinent data regarding the tetratogenicity of 3,3'-dichlorobenzidine were not located in the available literature. One study (Shabad et al., 1972) demonstrated that transplacental exposure of mice to 3,3'-dichlorobenzidine had effects on the growth of embryonic kidney cells in culture.

Because 3,3'-dichlorobenzidine has been demonstrated to be a carcinogen in animals, it is placed in EPA Group 2B, a probable human carcinogen. Therefore, inhalation and oral RfDs were not derived. A lack of pertinent data on the carcinogenicity of 3,3'-dichlorobenzidine following inhalation exposure precluded the derivation of an inhalation q_1^*. An oral q_1^* of 1.2 $(mg/kg/day)^{-1}$ was derived for humans from the study of Stula et al. (1975) in which female rats fed 3,3'-dichlorobenzidine in the diet (1000 ppm) over the course of a lifetime had a statistically significant increased incidence of mammary adenocarcinomas compared with controls. The levels of

3,3'-dichlorobenzidine in drinking water associated with increased lifetime risk at risk levels of 10^{-5}, 10^{-6} and 10^{-7} are $3x10^{-4}$, $3x10^{-5}$ and $3x10^{-6}$ mg/ℓ, respectively. An F factor of 8.4 $(mg/kg/day)^{-1}$, placing 3,3'-dichlorobenzidine in Potency Group 2, was also derived. Because 3,3'-dichlorobenzidine is categorized in EPA Group B2 and Potency Group 2, the compound has a MEDIUM hazard ranking under CERCLA. A medium hazard ranking is associated with an RQ of 10. An RQ based on systemic toxicity was also derived from a study by Stula et al. (1978) in which dogs given an oral dose of 3,3'-dichlorobenzidine (100 mg/day) over an extended period of time (i.e., up to 7.1 years) showed signs of liver toxicity in the form of elevated SGPT activities. This effect (liver toxicity) and this dose are associated with an RQ of 1000. Because 3,3'-dichlorobenzidine has been demonstrated to be carcinogenic in animals, however, the more conservative RQ of 10 derived from the carcinogenicity data is recommended.

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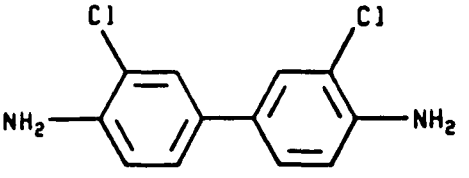
LIST OF ABBREVIATIONS

2-AAF	2-Acetylaminofluorene
BCF	Bioconcentration factor
BBN	N-butyl-N-(4-hydroxy butyl) nitrosamine
bw	Body weight
CAS	Chemical Abstract Service
CS	Composite score
DMBA	9,10-Dimethyl-1,2-benzanthracene
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
FANFT	N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide
HPLC	High performance liquid chromatography
K_{oc}	Soil sorption coefficient standardized with respect to organic carbon
K_{ow}	Octanol/water partition coefficient
LC_{50}	Concentration lethal to 50% of recipients (and all other subscripted concentration levels)
LD_{50}	Dose lethal to 50% of recipients
MED	Minimum effective dose
ppb	Parts per billion
ppm	Parts per million
RfD	Reference dose
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value
SGPT	Serum glutamic pyruvic transaminase
TLV	Threshold limit value
TWA	Time-weighted average
UV	Ultraviolet
w/w	Weight per weight

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

3,3'-Dichlorobenzidine is also known as DCB, C.I. 23060; 4,4'-diamino-3,3'-dichlorobiphenyl; and 3,3'-dichloro-(1,1'-biphenyl)-4,4'-diamine (IARC, 1982). The structure, CAS Registry number, empirical formula and molecular weight are as follows:



CAS Registry number: 91-94-1

Empirical formula: $C_{12}H_{10}Cl_2N_2$

Molecular weight: 253.1

1.2. PHYSICAL AND CHEMICAL PROPERTIES

3,3'-Dichlorobenzidine is a gray to purple crystalline solid at room temperature (Hawley, 1981). The reactions of this compound are similar to those of benzidine and other benzidine derivatives, e.g., formation of diazonium salts and acyl and alkyl derivatives (IARC, 1982; Ferber, 1978). 3,3'-Dichlorobenzidine is readily soluble in benzene, diethyl ether, ethanol and glacial acetic acid but almost insoluble in water (IARC, 1982). Selected physical properties are as follows:

Melting point:	133°C	Ferber, 1978
Boiling point:	422°C (estimated)	Neely and Blau, 1985
Vapor pressure at 25°C:	4.2×10^{-7} mm Hg (estimation based on the formula: TM = 0.5839 Tb)	Neely and Blau, 1985
Water solubility at 25°C:	3 mg/ℓ	Banerjee et al., 1980
Log K_{OW}:	3.51	Hansch and Leo, 1985

Conversion factor in air: 1 ppm = 0.0966 mg/m³ IARC, 1982

1.3. PRODUCTION DATA

3,3'-Dichlorobenzidine is produced commercially by alkaline reduction of o-chloronitrobenzene followed by rearrangement of the resulting hydrazo compound with hydrochloric acid to form the dihydrochloride salt (IARC, 1982). The dihydrochloride salt is the commercially available form of 3,3'dichlorobenzidine (IARC, 1982). The U.S. EPA TSCA Production File (U.S. EPA, 1977) contained no production data on 3,3'-dichlorobenzidine, but contained the following information on 3,3'-dichlorobenzidine dihydrochloride:

Company/Location	1977 Production/Import Volume
The Upjohn Co., North Haven, CT	confidential
Sun Chemical Corp., Cincinnati, OH (importer)	confidential
Bofors Lakeway Inc., Muskegon, MI	1-10 million pounds

SRI (1987) listed Bofors Nobel, Inc. in Muskegon, MI, and The Upjohn Co. in North Haven, CT, as the only current domestic manufacturers of 3,3'-dichlorobenzidine dihydrochloride. Domestic production volume data for recent years were not located in the available literature cited in Appendix A. During 1983, 1.104 million pounds of 3,3'-dichlorobenzidine base and salts was imported through principal U.S. custom districts (USITC, 1984).

1.4. USE DATA

Essentially 100% of all 3,3'-dichlorobenzidine (in the form of dihydrochloride salt) consumed in the United States is used as an intermediate for organic pigments (IARC, 1982; HSDB, 1987). At least 95 tetrazo dyes can be derived from 3,3'-dichlorobenzidine, but only 5

(Pigments Orange 13 and 14 and Pigments Yellow 12, 13 and 14) are currently used in the United States (Boyd et al., 1984; Shriner et al., 1978). These dyes are used in various paints, enamels and lacquers (Shriner et al., 1978). 3,3'-Dichloro- benzidine was also used in color tests for the presence of gold and used alone, or in blends with 4,4'-methylenebis-(2-chloroaniline) as a curing agent for liquid castable polyurethane elastomers (IARC, 1982).

1.5. SUMMARY

3,3'-Dichlorobenzidine (CAS number 91-94-1) is a gray to purple crystalline solid at room temperature (Hawley, 1981). It is available commercially in the form of its dihydrochloride salt (IARC, 1982). Commercial production involves alkaline reduction of o-nitrochlorobenzene, followed by rearrangement with hydrochloric acid to form 3,3'-dichlorobenzidine dihydrochloride (Ferber, 1978; IARC, 1982). Bofors Nobel, Inc. in Muskegon, MI, and The Upjohn Co. in North Haven, CT, are currently the only U.S. manufacturers of 3,3'-dichlorobenzidine dihydrochloride (SRI, 1987). 3,3'-Dichlorobenzidine dihydrochloride is imported into the United States; during 1983, 1.104 million pounds of 3,3'-dichlorobenzidine base and salts was imported through the principal U.S. customs districts (USITC, 1984). Essentially 100% of all 3,3'-dichlorobenzidine (in the form of its dihydrochloride salt) consumed in the United States is used as an intermediate for organic pigments (IARC, 1982; HSDB, 1987).

2. ENVIRONMENTAL FATE AND TRANSPORT

2.1. AIR

Based on a vapor pressure of 4.2×10^{-7} mm Hg at 25°C, 3,3'-dichlorobenzidine is expected to exist primarily in particulate form in the atmosphere.

2.1.1. Reaction with Hydroxy Radicals. The rate constant for the reaction of 3,3'-dichlorobenzidine vapor with photochemically generated hydroxyl radicals in the atmosphere was estimated to be $\sim 4 \times 10^{-11}$ cm^3/molecules-sec at 25°C using the method of Atkinson (1987). Assuming that the average ambient hydroxyl radical concentration is 5×10^5 molecules/cm^3, the hydroxyl reaction half-life for 3,3'-dichlorobenzidine vapor was estimated to be ~10 hours. Since only small amounts of 3,3'-dichlorobenzidine released to the atmosphere are expected to exist in the vapor phase, the environmental significance of this reaction would be limited.

2.1.2. Reaction with Ozone. 3,3'-Dichlorobenzidine is not susceptible to oxidation by reaction with ozone molecules in the atmosphere (U.S. EPA, 1987a).

2.1.3. Photolysis. 3,3'-Dichlorobenzidine in methanol, ethanol or water exhibits strong absorption of UV light wavelength in the environmentally significant range (wavelength 290-340nm) (Sadtler, n.d.; Callahan et al., 1979). 3,3'-Dichlorobenzidine, adsorbed onto silica gel, underwent 41.2% degradation (based on volatile compounds and CO_2 evolved in the photodegradation process) when irradiated with light (wavelengths >290 nm) for 17 hours (Freitag et al., 1985). This information combined with the observed rapid photolysis of 3,3'-dichlorobenzidine in water suggests that 3,3'-dichlorobenzidine in both particulate and vapor form would rapidly photolyze in the atmosphere.

2.2. WATER

2.2.1. Hydrolysis. Chemical hydrolysis of 3,3'-dichlorobenzidine in water is not expected to be environmentally significant (Mabey et al., 1981; U.S. EPA, 1981).

2.2.2. Oxidation. Pertinent data regarding the oxidation of 3,3'-dichlorobenzidine in natural waters were not located in the available literature cited in Appendix A. Unsubstituted benzidine is rapidly oxidized by iron (+3) and several other naturally occurring cations that can be found in environmental waters as solvated cations, as complexes of humic acids and as structural components of microcrystalline clays. Whether or not 3,3'-dichlorobenzidine would be oxidized rapidly in a similar manner depends upon its ionization potential in relation to the ionization potential of benzidine itself. Because of the two chlorine substituents on the aromatic rings of 3,3'-dichlorobenzidine, this compound would have a lesser tendency to lose an electron (i.e., to oxidize) than unsubstituted benzidine. Nevertheless, it has been speculated that oxidation by metal cations and other environmental electron acceptors may contribute to the degradation of 3,3'-dichlorobenzidine in sediments (Callahan et al., 1979).

2.2.3. Photolysis. Dilute (10^{-5} M) aqueous solutions of 3,3'-dichlorobenzidine in quartz tubes at neutral pH were exposed to noonday summer sunlight at Syracuse, NY. Irradiation resulted in a half-life of ~90 sec (Banerjee et al., 1978). 3,3'-Dichlorobenzidine photodegraded to monochlorobenzidine, benzidine and a number of brightly colored water-insoluble materials. The same intermediate products were formed upon photolysis of aqueous solutions under acidic conditions and aqueous solutions treated with chlorine water (Banerjee et al., 1978). The photolysis half-life for 3,3'-dichlorobenzidine in organic solvents is markedly longer than in aqueous solutions, and the mechanism of dechlorination does not appear to

involve simple carbon-chlorine bond homolysis. Reduced photo-reactivity in nonaqueous solutions, along with the high K_{ow} of 3,3'-dichlorobenzidine, might lead to enhanced stability of this compound in water contaminated with hydrocarbons (Banerjee et al., 1978).

2.2.4. Microbial Degradation. [^{14}C]-3,3'-dichlorobenzidine was incubated (21°C in the dark) in natural water samples obtained from a eutrophic lake and a mesotrophic lake. After a 1-month incubation period, 75% of the original 3,3'-dichlorobenzidine was detected when assayed by HPLC and no metabolites were detected (Appleton et al., 1978; Sikka et al., 1978). Therefore, 3-3'-dichlorobenzidine appears to resist biodegradation by aquatic microbes. Sikka et al. (1978) speculated that loss of the compound was primarily the result of adsorption to sediments or accumulation by aquatic organisms. [The BCF for 3,3'-dichlorobenzidine in activated sludge is 3100 (Freitag et al., 1985).] In a biodegradation screening study using activated sewage as seed, 3,3'-dichlorobenzidine at an initial concentration of 3 mg/ℓ underwent 9-99% degradation in 28 days when yeast extract was present at concentrations of 10-400 mg/ℓ. Extent of degredation was dependent on the concentration of yeast extract and no degradation was observed in this absence of this additional nutrient (Brown and Laboureur, 1983). As a result, 3,3'-dichlorobenzidine was considered to be "inherently biodegradable" rather than "readily biodegradable". Brown and Laboureur (1983) suggested that the yeast extract may have provided growth factors necessary for the breakdown of this amine or it might have been acting as a readily degradable food source, building up a large concentration of active bacteria, which were then able to break down 3,3'-dichlorobenzidine. In another biodegradation screening study, 2.7% degradation (based on $\%CO_2$ evolved) of 0.05 mg/ℓ 3,3'-dichlorobenzidine was observed after 5 days incubation in activated sludge (Freitag et al., 1985).

2.2.5. Volatilization. Henry's Law constant for 3,3'-dichlorobenzidine was estimated to be ~$5x10^{-8}$ atm-m^3/mol at 25°C based on a water solubility of 3 mg/ℓ at 25°C and an estimated vapor pressure of $4.2x10^{-7}$ mm Hg at 25°C. This value for Henry's Law constant indicates that this compound is essentially nonvolatile and that volatilization would not be a significant fate process in water (Lyman et al., 1982).

2.2.6. Bioaccumulation. 3,3'-Dichlorobenzidine was found to be rapidly and significantly bioaccumulated by fish and algae. Appleton and Sikka (1980) exposed bluegill sunfish, Lepomis macrochirum, to water containing 5 and 100 µg/ℓ radiolabelled 3,3'-dichlorobenzidine until reaching equilibrium (4-7 days). Based on total ^{14}C residues found at equilibrium, the apparent BCF of 3,3'-dichlorobenzidine was determined to be 495-507 in whole fish, 114-170 in the edible portion and 814-856 in head and viscera. Appleton and Sikka (1980) suggested the possibility that either enterohepatic circulation of 3,3'-dichlorobenzidine and metabolites or covalent binding to lipoprotein may account for some of the residual radioactivity detected. In another study, a BCF of 610 was found in Golden ide, Leuciscus idus melanotus, exposed to 50 µg/ℓ radiolabelled 3,3'-dichlorobenzidine for 3 days; a BCF of 940 was found in a green algae, Chlorella fusca, exposed to 50 µg/ℓ radiolabelled 3,3'-dichlorobenzidine for 1 day (Freitag et al., 1985).

2.2.7. Adsorption. The relative distribution ratio of 3,3'-dichlorobenzidine between natural aquatic sediments and water at pH 5-7 was found to range between 26.7 and 128 (Appleton et al., 1978). Adsorption to sediments was inhibited by 30-50% under alkaline conditions (pH 9). Equilibration of 3,3'-dichlorobenzidine between water and sediment was generally achieved within 24 hours and desorption from sediments was very low. Based on the behavior of other aromatic amines it was speculated that initial adsorption

of 3,3'-dichlorobenzidine to sediments was probably the result of physical adsorption processes, which was then followed by covalently binding with the sediment. Irreversible binding with sediment would explain the increased resistance of 3,3'-dichlorobenzidine to desorption and extraction observed over time (Sikka et al., 1978).

2.3. SOIL

2.3.1. Hydrolysis. 3,3'-Dichlorobenzidine contains no hydrolyzable functional groups (Callahan et al., 1979); therefore, this compound is not expected to undergo chemical hydrolysis in soil.

2.3.2. Oxidation. Unsubstituted benzidine is rapidly oxidized by Fe^{+3}, Al^{+3}, Cu^{+3} and a few other naturally occurring cations, which can be found in soil as part of humic acid complexes and microcrystalline clays (Callahan et al., 1979; Demirjian et al., 1987). Whether or not 3,3'-dichlorobenzidine would be oxidized rapidly in a similar manner is not certain; however, the possibility exists that 3,3'-dichlorobenzidine may be oxidized by cationic components of soil (see Section 2.2.2.).

2.3.3. Photolysis. Given that 3,3'-dichlorobenzidine photogrades rapidly in water and when adsorbed onto silica gel (see Sections 2.1.3. and 2.2.3.) it is also expected to photodegrade rapidly on soil surfaces.

2.3.4. Microbial Degradation. A Brookston clay loam soil was mixed with ^{14}C-3,3'-dichlorobenzidine (total concentrations of 4 or 40 ppm were achieved in 2 separate batches) and incubated in the dark under aerobic conditions for 32 weeks or under anaerobic conditions for 1 year. Cumulative $^{14}CO_2$ production was ~2% after 32 weeks incubation in aerobic soil containing 4 and 40 ppm 3,3'-dichlorobenzidine. No radioactive $^{14}CH_4$ or $^{14}CO_2$ was detected in the headspace gas above the anaerobic soil after 1 year of incubation (Boyd et al., 1984).

2.3.5. Adsorption. The K_{oc} for 3,3'-dichlorobenzidine was experimentally determined to be 16,300 in Brookston clay loam and 33,700 in Rubicon sand (Boyd et al., 1984). These K_{oc} values indicate that strong physical adsorption of 3,3'-dichlorobenzidine to soil takes place. 3,3'-Dichlorobenzidine has also been shown to bind strongly to soil. When radiolabelled 3,3'-dichlorobenzidine (at 4 and 40 ppm concentration) were added to Brookston clay loam, >50% of the ^{14}C was nonextractable after the first several weeks of incubation. In general, at least 50% of the aromatic amines became bound to soil 1 day to 1 week. Aromatic amines such as 3,3'-dichlorobenzidine are believed to form covalent linkages with humic substances in soil, thus immobilizing them in soil. Two different mechanisms have been proposed for the chemical binding of amines to soil (Boyd et al., 1984). Rapid reversible binding of primary amines with humate carbonyls is believed to result in imine formation. Subsequent slow irreversible reaction thought to represent 1,4-addition to quinone rings is believed to occur. This addition would result in an amino-substituted quinone. The amine group can be further converted to heterocyclic nitrogen, which is present in the humate structure. These reactions may proceed enzymatically and chemically (Boyd et al., 1984).

2.3.6. Volatilization. Volatilization of 3,3'-dichlorobenzidine from soil surfaces is expected to be negligible since this compound binds/adsorbs strongly to soil and has a relatively low Henry's Law constant (see Sections 2.2.5. and 2.3.3.). At the end of 1 year incubation of labeled 3,3'-dichlorobenzidine and soil, essentially all the original ^{14}C still remained in the soil, demonstrating that volatile losses of 3,3'-dichlorobenzidine or its metabolites had not occurred in this system (Boyd et al., 1984).

2.4. SUMMARY

In the atmosphere, 3,3'-dichlorobenzidine is expected to exist primarily in the particulate form. 3,3'-Dichlorobenzidine, in both vapor and particulate form, is expected to undergo rapid photolysis in the atmosphere. Reaction of 3,3'-dichlorobenzidine vapor with photochemically generated hydroxyl radicals ($t_{1/2}$=10 hours) (Atkinson, 1987) may be a minor removal mechanism. In water, this compound would undergo rapid photodegradation ($t_{1/2}$=90 sec) in the surface layers of water (Banerjee et al., 1978). This compound photodegrades to monochlorobenzidine, benzidine and a number of brightly colored water-insoluble compounds (Banerjee et al., 1978). Beyond the reach of light penetration, this compound would rapidly adsorb to sediment and particulate matter where it is tightly bound. Adsorption is expected to proceed initially by a rapid, but reversible, physical adsorption process followed by much slower irreversible covalent bonding (Appleton et al., 1978; Sikka et al., 1978). It has also been speculated that 3,3'-dichlorobenzidine may be oxidized by naturally occurring cations such as, Fe^{+3} found in sediments (Callahan et al., 1979). Rapid uptake and bioaccumulation in aquatic organisms is also expected to occur (Appleton and Sikka, 1980; Freitag et al., 1985). Volatilization, microbial degradation and chemical hydrolysis are not expected to be important fate processes in water. In soil, 3,3'-dichlorobenzidine is expected to adsorb tightly to soil, and over time, irreversibly bind with humates in the soil (Boyd et al., 1984). It has also been speculated that oxidation by reaction with cationic constituents of soil such as, Fe^{+3} and Cu^{+3} may also occur (Callahan et al., 1979; Demirjian et al., 1987). If exposed to sunlight on soil surfaces, 3,3'-dichlorobenzidine is expected to photodegrade rapidly. Volatilization and microbial degradation are not expected to be significant fate processes.

3. EXPOSURE

Exposure to 3,3'-dichlorobenzidine is most likely to occur in occupational settings, particularly where this compound is manufactured or where 3,3'-dichlorobenzidine-based dyes are manufactured or used (HSDB, 1987). In 1973, 18 U.S. companies were using 3,3'-dichlorobenzidine and 166-250 workers were potentially exposed to this compound (IARC, 1982). The general public may be exposed to 3,3'-dichlorobenzidine during use of paints, pigments or enamels derived from this compound. 3,3'-Dichlorobenzidine occurs at a level of ~20 ppm in most pigments (Lapp et al., 1981). The most probable routes of exposure are inhalation of dusts or mists and dermal contact (HSDB, 1987).

3.1. WATER

3,3'-Dichlorobenzidine has been detected in samples of surface water, sediment, fish and industrial effluent collected throughout the United States. Monitoring data are provided in Table 3-1.

3.2. FOOD

Pertinent monitoring data regarding the presence of 3,3'-dichlorobenzidine in food were not located in the available literature cited in Appendix A.

3.3. INHALATION

Limited data were available concerning the detection of 3,3'-dichlorobenzidine in air. Results of a 1970 Japanese study on worker exposure to 3,3'-dichlorobenzidine in a pigment manufacturing plant revealed that the concentration of 3,3'-dichlorobenzidine in the air reached a level of 25 $\mu g/m^3$ (2 ppb) within 10 minutes of charging reaction vessels and dropped to 2 $\mu g/m^3$ (0.2 ppb) within 20 minutes (IARC, 1982).

TABLE 3-1

Monitoring Data for 3,3'-Dichlorobenzidine in Various Media

Sample Type	Location	Number of Samples	Concentration	Reference
Total water	United States	7334	46 µg/ℓ (mean)	U.S. EPA, 1987b
Sediment[a]	United States	952	3222 µg/kg dry wt. basis (mean)	U.S. EPA, 1987b
Sediment[a]	United States	68	0.026 mg/kg wet wt. basis (mean)	U.S. EPA, 1987b
Fish tissue	United States	650	5.8 mg/kg wet wt. basis (mean)	U.S. EPA, 1987b
Surface water	Buffalo River, Cayuhoga River, St. Joseph's River	NR	not detected[b]	Great Lakes Water Quality Board, 1983
Surface water	Sumida River[c] (Japan)	NR	qualitatively identified	IARC, 1982
Sediment/soil/ water	Love Canal (Niagara Falls, NY); 1980	NR	qualitatively identified	Hauser and Bromberg, 1982
Purge wells and seepage water	near a waste disposal lagoon receiving waste from the manufacture of 3,3'-dichlorobenzidine	NR	0.13-0.27 mg/ℓ	IARC, 1982

TABLE 3-1 (cont.)

Sample Type	Location	Number of Samples	Concentration	Reference
Treated effluent from coal mining	United States	52 (2% pos.)	3 µg/ℓ	U.S. EPA, 1981
Treated effluent from nonferrous metal manufacturing	United States	18	0.2 µg/ℓ (mean)	U.S. EPA, 1981
Treated effluent[d]	United States	NR	not detected[b]	U.S. EPA, 1981
Urban runoff[e]	15 United States cities	86	not detected	Cole et al., 1984

[a]Sediment samples were analyzed on either a wet or dry weight basis.

[b]Detection limit not reported

[c]This River receives wastewater from several dye and pigment factories.

[d]Treated effluent from leather tanning and finishing, aluminum forming, battery manufacturing, coil coating, foundaries, porcelain enameling, gum and wood chemicals, pharmaceutical manufacturing, organic chemicals manufacturing/plastics, pulp and paperboard mills, rubber processing, steam electric power plants, timber products processing.

[e]U.S. EPA Nationwide Urban Runoff Program Findings as of July 1982.

NR = Not reported

3.4. DERMAL

Pertinent monitoring data regarding dermal exposure to 3,3'-dichlorobenzidine were not located in the available literature cited in Appendix A.

3.5. SUMMARY

Exposure to 3,3'-dichlorobenzidine is most likely to occur in occupational settings, particularly where this compound is manufactured or where 3,3'-dichlorobenzidine-based dyes are manufactured or used (HSDB, 1987). The general public may be exposed to low levels of 3,3'-dichlorobenzidine during use of paints, pigments or enamels derived from this compound. The most probable routes of exposure are inhalation of dusts or mists containing this compound and dermal contact (HSDB, 1987). 3,3'-Dichlorobenzidine has been detected in samples of surface water, sediment, fish and industrial effluent (U.S. EPA, 1981, 1987b; IARC, 1982; Hauser and Bromberg, 1982). This compound has not been detected in urban runoff samples collected as part of the U.S. EPA Nationwide Urban Runoff Program (Cole et al., 1984). The U.S. EPA STORET Data Base indicates that the mean concentrations of 3,3'-dichlorobenzidine in total water, sediment (on either wet or dry weight basis) and fish tissue samples collected throughout the United States are 46 µg/ℓ, 3222 µg/kg dry wt. of sediment, 0.026 mg/kg wet wt. of sediment, and 5.8 mg/kg wet wt. of fish tissue, respectively (U.S. EPA, 1987b).

4. AQUATIC TOXICITY

There are few data regarding the aquatic toxicity of 3,3'-dichlorobenzidine. A study by Appleton et al. (1978) indicated that in bluegills, Lepomis macrochirus, toxic levels of 3,3'-dichlorobenzidine accumulated before a 3,3'-dichlorobenzidine equilibrium was reached between water and fish. There were many mortalities when the whole body residues of ^{14}C-3,3'-dichlorobenzidine exceeded 150 ppm.

5. PHARMACOKINETICS

5.1. ABSORPTION

The absorption of 3,3'-dichlorobenzidine following administration of the compound by relevant routes (i.e., oral and inhalation) has not been studied extensively. Hsu and Sikka (1982) reported that 3,3'-dichlorobenzidine is absorbed readily following oral administration of the compound to rats. The kinetics of the appearance of radio-activity in the plasma monitored in rats following a single oral dose of radiolabeled 3,3'-dichlorobenzidine (40 mg/kg) in DMSO. Total plasma radioactivity reached a peak (9.4 µg 3,3'-dichlorobenzidine equivalents/mℓ) 8 hours after dosing and then declined in a biphasic manner. The appearance and disappearance of total radioactivity in the plasma could be described by a first-order absorption process with a half-life of 1.59 hours for the appearance and 1.68 and 33 hours, respectively, for the two elimination process. The kinetic pattern of unchanged 3,3'-dichlorobenzidine in plasma was similar to that for total radioactivity but reached a peak (1.25 µg 3,3'-dichlorobenzidine/mℓ) 4 hours after dosing and after which time it declined in a biphasic manner. The absorption of unchanged 3,3'-dichlorobenzidine was also first-order with a reported half-life of 2.12 hours for the appearance and 5.58 and 13.59 hours for the disappearance processes.

Data regarding the excretion of 3,3'-dichlorobenzidine and metabolites in the urine, bile and feces of rats given an oral dose of 40 mg 3,3'-dichlorobenzidine/kg indicated that $\geq$90% of the administered dose was absorbed (Hsu and Sikka, 1982).

Pertinent data regarding the absorption of 3,3'-dichlorobenzidine following inhalation exposure in humans or animals were not located in the available literature.

There appears to be significant absorption of 3,3'-dichlorobenzidine following dermal exposure to the compound in both humans and animals. An early study (Meigs et al., 1954) of occupational exposure to 3,3'-dichlorobenzidine indicated that the skin was the principal route of entry of this compound in exposed workers. Suskind (1983) also reported that absorption of 3,3'-dichlorobenzidine through the skin is the major route of entry into the body. The dermal absorption of 3,3'-dichlorobenzidine in rats was studied by Shah and Guthrie (1983), who applied 0.2 mℓ of ^{14}C-3,3'-dichlorobenzidine in acetone (total dose 1 mg/kg) to the shaved backs of Fisher 344 rats. The amount of 3,3'-dichlorobenzidine absorbed from the application site after 24 hours was ~50% of the applied dose (half-life of disappearance of radioactivity from the application site was estimated to be 24.1 hours).

5.2. DISTRIBUTION

The distribution of radioactivity in rats given either single or multiple oral doses of ^{14}C-radiolabeled 3,3'-dichlorobenzidine was studied by Hsu and Sikka (1982). Rats were given a single oral dose of either 6.4 or 40 mg 3,3'-dichlorobenzidine/kg, and various tissues were analyzed for radioactivity 24 or 96 hours after dosing. At both dose levels and at both sacrifice times, the liver, lung and kidney were the principal sites of distribution of 3,3'-dichlorobenzidine-derived radioactivity. At the 40 mg 3,3'-dichlorobenzidine/kg dose level, the concentration of radioactivity in the principal organs of accumulation reached maximum levels 12-16 hours following dosing; maximum levels for the various organs were liver, 53.4 µg 3,3'-dichlorobenzidine equivalents/g tissue; kidney, 36.9 µg 3,3'-dichlorobenzidine equivalents/g tissue; and lung, 16.1 µg 3,3'-dichlorobenzidine equivalents/g tissue. The measured half-lives for the appearance of radioactivity in these tissues were 3.32, 2.5 and 5.41 hours, respec-

tively, for the liver, lung and kidney. In the same study, multiple oral dosing of rats with 3,3'-dichlorobenzidine (6 mg/kg/day for 6 days) led to tissue distributions of radioactivity similar to those observed after single doses (i.e., the highest concentrations of radioactivity were found in the liver and kidney). The tissue concentrations of radioactivity were, however, 3-4 times higher in rats given multiple doses. Multiple oral dosing with 3,3'-dichlorobenzidine did not result in substantial retention of radioactivity, and Hsu and Sikka (1982) concluded that the compound had a fairly low tendency to accumulate in the body.

The distribution of radioactivity in the tissues of rats following dermal application of a solution of 3,3'-dichlorobenzidine in acetone (total 3,3'-dichlorobenzidine dose of 1 mg/kg) was studied by Shah and Guthrie (1983). For most tissues the amount of radioactivity recovered 24 hours after 3,3'-dichlorobenzidine application was <0.1% of the dose. The liver, however, showed the highest level of radioactivity, ~4% of the applied dose.

5.3. METABOLISM

The limited data available regarding the metabolism of 3,3'-dichlorobenzidine do not provide sufficient information to suggest a metabolic scheme for the compound. Troll (n.d.) was unable to detect ortho-hydroxy metabolites of 3,3'-dichlorobenzidine in the urine of humans and dogs after oral dosing and Shriner et al. (1978) suggested that the chlorine constituents of 3,3'-dichlorobenzidine probably block ring hydroxylation reactions for both electronic and steric reasons. Hsu and Sikka (1982), however, have provided evidence that 3,3'-dichlorobenzidine is metabolized extensively in rats. These investigators found at least five metabolites of 3,3'-dichlorobenzidine in ether extracts of urine and bile samples from rats 24 hours after treatment with an oral dose of radiolabeled 3,3'-dichlorobenzidine (40 mg/kg). Although the metabolites were not identified, it was

determined that only 9 and 16% of the total radioactivity excreted in the urine and bile, respectively, could be accounted for by the parent compound. Therefore 3,3'-dichlorobenzidine has been demonstrated to be metabolized extensively following absorption in this study.

Kellner et al. (1973) reported the presence of a compound with chromatographic properties similar to mono-N-acetyl 3,3'-dichlorobenzidine in the urine of monkeys dosed intravenously with ^{14}C-labeled 3,3'-dichlorobenzidine (0.2 mg/kg). Aksamitnaia (1959) reported the appearance of four transformation products in the urine of rats given repeated oral doses of 3,3'-dichlorobenzidine over a period of 7.5-8.5 months. The products of 3,3'-dichlorobenzidine metabolism, identified only by paper chromatography, were tentatively identified as benzidine and some possible glucuronide conjugates.

Several studies are available regarding the in vitro metabolism of 3,3'-dichlorobenzidine. Cytochrome c was reported to be incapable of oxidizing 3,3'-dichlorobenzidine, whereas benzidine and several other derivatives were oxidized (Hirai and Yasuhira, 1972). Studies of the binding of radiolabeled 3,3'-dichlorobenzidine to calf thymus DNA indicated that 3,3'-dichlorobenzidine reacted with DNA both in the absence and presence of rat liver S-9 fraction. Binding in the presence of S-9 was considerably higher than that observed in its absence (Bratcher and Sikka, 1982).

5.4. EXCRETION

The elimination of 3,3'-dichlorobenzidine and 3,3'-dichlorobenzidine-derived radioactivity from the plasma and principal organs of distribution (liver, lung and kidney) was characterized in rats treated with single oral doses of ^{14}C-labeled 3,3'-dichlorobenzidine (Hsu and Sikka, 1982). The disappearance of both the parent compound and total radioactivity from the

plasma of 3,3'-dichlorobenzidine-treated rats was biphasic. The elimination half-lives for total radioactivity from the plasma were 1.68 and 33 hours for the fast and slow phases, respectively. The corresponding values for the fast and slow phases of elimination of the parent compound from the plasma were 5.58 and 13.59 hours, respectively. The disappearance of radioactivity from the major tissues of distribution was also biphasic in nature. Half-lives for the slow and fast phases were liver, 5.78 and 77 hours; lung, 3.85 and 43.3 hours; and kidney, 7.14 and 138.6 hours.

The bile appears to be a significant route of excretion of 3,3'-dichlorobenzidine and metabolites. Preliminary experiments with rats indicated that >90% of the administered radioactivity was excreted via the urine and feces 72 hours after receiving single oral doses of radiolabeled 3,3'-dichlorobenzidine (40 mg/kg) (Hsu and Sikka, 1982). Of this 3,3'-dichlorobenzidine-derived radioactivity, 64.9% was found in the feces and 27.7% of the administered radioactivity was found in the urine. To determine the source of the radioactivity appearing in the feces, further experiments were performed using bile duct-cannulated rats given oral doses of ^{14}C-labeled 3,3'-dichlorobenzidine. Cumulative excretion of radioactivity was monitored in the urine, feces and bile for up to 72 hours after dosing. Excretion of radioactivity in the urine, bile and feces again approached 90% of the administered radioactivity; however, ~65% of the administered dose was found in the bile within 24 hours of dosing compared to 48% in the intact rats. Excretion of radioactivity in the urine and feces decreased to <10% of the administered dose for each route compared to 27.7% and 64.9%, respectively, in intact rats. Hsu and Sikka (1982) concluded that biliary excretion is a significant route of elimination for 3,3'-dichlorobenzidine and its metabolites.

Support for the importance of hepatobiliary excretion in the elimination of 3,3'-dichlorobenzidine is provided by a study by Kellner et al. (1973). Following intravenous administration of a radiolabeled dose of 3,3'-dichlorobenzidine to rhesus monkeys, most of the administered dose was recovered from the bile, intestine and liver within 14 hours of treatment.

Troll (n.d.) reported that in humans 3,3'-dichlorobenzidine is excreted largely is the feces. Several investigators have also reported detectable quantities of 3,3'-dichlorobenzidine in the urine of 3,3'-dichlorobenzidine processing and manufacturing workers (Meigs et al., 1954; Singal and Lee, 1985; London and Boiano, 1986).

5.5. SUMMARY

The absorption of 3,3'-dichlorobenzidine following administration of the compound by relevant routes (inhalation or oral exposure) has not been studied extensively. Hsu and Sikka (1982) reported that 3,3'-dichlorobenzidine is rapidly absorbed and extensively distributed following oral administration of the compound to rats. Meigs et al. (1954) and Suskind (1983) reported that the skin is the most significant route of entry of 3,3'-dichlorobenzidine into the body in cases of occupational exposure. The half-life of dis- appearance of a topically applied 3,3'-dichlorobenzidine dose from the shaved backs of rats was determined to be 24.1 hours (Shah and Guthrie, 1983).

Following a single oral dose of radiolabeled 3,3'-dichlorobenzidine to rats, the principal organs in which radioactivity was found were the liver, kidneys and lungs (Hsu and Sikka, 1982). Multiple oral 3,3'-dichlorobenzidine dosing led to tissue levels of radioactivity 3-4 times higher than the levels observed following a single oral dose, but a multiple dosing schedule did not result in substantial retention of radioactivity (Hsu and Sikka, 1982).

The extent of metabolism and the pathways of 3,3'-dichlorobenzidine metabolism are not clear. Ring hydroxylation products of 3,3'-dichlorobenzidine were not found in the urine of humans and dogs given an oral 3,3'-dichlorobenzidine dose (Troll, n.d.), and Shriner et al. (1978) suggested that chlorination of benzidine blocks ring hydroxylation reactions of 3,3'-dichlorobenzidine for both electronic and steric reasons. Hsu and Sikka (1982), however, provided evidence that 3,3'-dichlorobenzidine is metabolized extensively in rats.

Possible metabolites of 3,3'-dichlorobenzidine, tentatively identified by chromotographic procedures, include mono-N-acetyl 3,3'-dichlorobenzidine in the urine of monkeys (Kellner et al., 1973) and benzidine and some possible glucuronide conjugates in the urine of rats (Aksamitnaia, 1959).

Elimination of both radiolabeled 3,3'-dichlorobenzidine and total radioactivity from the plasma of orally dosed rats was biphasic (Hsu and Sikka, 1982). Similarly, biphasic mode of elimination of total radioactivity was observed in the principal organs of distribution (liver, lung and kidney) (Hsu and Sikka, 1982).

The bile appears to be a significant route of excretion for both 3,3'-dichlorobenzidine and its metabolites. Experiments using rats indicated that ~90% of the administered radioactivity is excreted in the urine and feces following an oral dose of 3,3'-dichlorobenzidine (Hsu and Sikka, 1982). Approximately 65% of the administered radioactivity was excreted in the feces and the major source of the radioactivity found in the feces originated from the bile. Hepatobiliary excretion of 3,3'-dichlorobenzidine and its metabolites also occurred in rhesus monkeys (Kellner et al., 1973).

The fecal route appears to be the most significant route of elimination of 3,3'-dichlorobenzidine and metabolites in humans (Troll, n.d.), 3,3'-dichlorobenzidine has also been reported to be present in the urine of occupationally-exposed individuals (Meigs et al., 1954; London and Boiano, 1986; Singal and Lee, 1985).

6. EFFECTS

6.1. SYSTEMIC TOXICITY

6.1.1. Inhalation Exposures. Pertinent data regarding the systemic toxicity of 3,3'-dichlorobenzidine following subchronic or chronic inhalation exposure in either animals or humans were not located in the available literature cited in Appendix A.

6.1.2. Oral Exposures.

6.1.2.1. SUBCHRONIC -- Pertinent data regarding the systemic toxicity of 3,3'-dichlorobenzidine following subchronic oral exposure in either animals or humans were not located in the available literature cited in Appendix A.

6.1.2.2. CHRONIC -- Six female beagle dogs were given 3,3'-dichlorobenzidine (100 mg/day, ~100% pure) by capsule 3 times/week for 6 weeks, then 5 times/week continuously for an additional 7 years (total duration = 7.1 years) (Stula et al., 1978). Six untreated female beagle dogs served as controls and were sacrificed after 8.3-9.0 years. Urine and blood samples were taken once before the test began and then approximately every 6 months during the remainder of the test. At sacrifice, a complete necropsy and histological examination were performed on all dogs. All six 3,3'-dichlorobenzidine-treated dogs had elevated SGPT activities during the first 3 years of treatment. SGPT activities remained elevated in two of four dogs that survived the full treatment period (7.1 years).

6.1.3. Other Relevant Information. The oral LD_{50} of in albino rats (sex and strain not specified) was reported to be ~7 g/kg bw (ACGIH, 1986) for 3,3'-dichlorobenzidine and 3.82 g/kg bw for dihydrochloride salt of 3,3'-dichlorobenzidine.

Human fibroblast cell cultures were found to be more sensitive than hamster fibroblast cell cultures to the cytotoxic effects due to 3,3'-dichlorobenzidine exposure (Casto, 1983). The LC_{50} following 18 hours of exposure to 3,3'-dichlorobenzidine was determined to be 250 µg/mℓ for hamster cells and 50 µg/mℓ for human cells.

6.2. CARCINOGENICITY

6.2.1. Inhalation. Pertinent data regarding the carcinogenicity of 3,3'-dichlorobenzidine following inhalation exposure in either animals or humans were not located in the available literature cited in Appendix A.

6.2.2. Oral. One of the first demonstrations of the carcinogenicity of 3,3'-dichlorobenzidine following oral administration in rats was a study by Pliss (1959). Fifteen female and 35 male outbred Rappolovo rats were fed 3,3'-dichlorobenzidine in a paste (45.3% 3,3'-dichlorobenzidine, 50% water and 4.7% unspecified impurities) in an amount that provided a dose of 10-20 mg/day. The paste was administered 6 days/week for 12 months (total dose of 4.5 g 3,3'-dichlorobenzidine/rat) and the rats were observed for life. Control animals (130 rats) were injected with either octadecylamine or methylstearylamine and observed for 23 months. The numbers of rats that survived were: 34 at 6 months, 29 at the time of appearance of the first tumor (11 months) and 27 at 12 months. Twenty-three out of 50 3,3'-dichlorobenzidine-treated rats developed tumors. There were seven tumors of the Zymbal gland, three skin tumors, seven mammary gland tumors, two adenocarcinomas of the ileum, three bladder tumors, three tumors of the haematopoietic system, two tumors of the connective tissue, two salivary gland tumors, one liver tumor and one thyroid tumor. Incidences, expressed as the number of rats with a particular tumor type per number of rats examined, were not provided. No tumors were found in the control group. However, the lack of adequate controls was noted in IARC (1982).

Fifty female and fifty male ChR-CD rats were fed 3,3'-dichlorobenzidine in the diet (1000 ppm 3,3'-dichlorobenzidine, purity and impurities unspecified) for an average period of 349 days (range of 143-488 days) for females and 353 days (range of 118-486 days) for males (Stula et al., 1975). Control rats (50 male and 50 female) were fed a standard diet and were maintained under observation for up to ~2 years. Six 3,3'-dichlorobenzidine-treated rats/sex were sacrificed at 12 months and were not included in the tumor analysis. Of the remaining forty-four 3,3'-dichlorobenzidine-treated rats of each sex, statistically significant ($p<0.05$) increases in tumor incidences over those observed in controls were reported (Table 6-1). 3,3'-Dichlorobenzidine-treated male rats had significantly increased incidences of granulocytic leukaemias, mammary adenocarcinomas and Zymbal gland carcinomas. 3,3'-Dichlorobenzidine-treated female rats had a significantly increased incidence of mammary adenocarcinomas.

The ability of 3,3'-dichlorobenzidine dihydrochloride to produce mammary tumors in young female Sprague-Dawley rats (40 days old) was investigated by Griswold et al. (1968). A group of 20 rats were given 10 doses of 3,3'-dichlorobenzidine dihydrochloride by gastric intubation every 3 days, which was a total administered 3,3'-dichlorobenzidine dose of 300 mg/rat over a 30-day period. Rats were observed for 9 months following treatment. Fourteen 3,3'-dichlorobenzidine-treated rats survived to the end of the 9-month observation period. Negative controls were administered only with the vehicle (sesame oil) and positive controls received a single dose of 18 mg DMBA. At necropsy and histological examination, none of the 3,3'-dichlorobenzidine-treated rats had mammary tumors, while the incidence of mammary tumors was 100% in the DMBA controls and 3% in the negative control group.

TABLE 6-1

Incidence of Tumors in Female Beagle Dogs Given 3,3-Dichlorobenzidine (~100% pure) Orally by Capsule[a]

Dose	Duration of Study (years)	Target Organ	Tumor Type	Tumor Incidence[b] (p value)[c]
100 mg/day, 3 times/week for 6 weeks, followed by 5 times/week for 7 years	≤7.1	liver	carcinoma	4/5 (p<0.025)
		urinary bladder	papillary transitional cell carcinoma	5/5 (p<0.025)
0 (untreated controls)	8.3-9.0	liver or urinary bladder	NA	0/6

QUALITY OF EVIDENCE

Strength of study: Compound was administered by a relevant route of exposure for a sufficient duration for tumor development.

Weakness of the study: Only six dogs were started on the study and only five survived to be at risk for late appearing tumors; only one dose level was administered to only one sex.

Overall adequacy: Adequate

[a]Source: Stula et al., 1978

[b]Tumor incidence expressed as number of animals with tumors/number of animals necropsied

[c]p value is for Fisher Exact test (one tail)

NA = Not applicable

Six female beagle dogs were given a daily oral dose of 100 mg 3,3'-dichlorobenzidine (~100% pure) by administration of a capsule 3 times/week for 6 weeks, then 5 times/week for up to an additional 7 years (total duration equal to 7.1 years) (Stula et al., 1978). Six untreated beagle dogs served as controls. A complete necropsy and histological examination were performed on all dogs at the end of the test period. One 3,3'-dichlorobenzidine-treated dog, sacrificed in extremis after 3.5 years, had no tumors. Another 3,3'-dichlorobenzidine-treated dog, sacrificed in extremis after 6.6 years, developed an undifferentiated carcinoma of the liver and a papillary transitional cell carcinoma of the urinary bladder. Of the four 3,3'-dichlorobenzidine-treated dogs that survived the full test period of 7.1 years, all had papillary transitional cell carcinomas of the urinary bladder and three had hepatocellular carcinomas. None of the six control dogs had liver or urinary bladder tumors (Table 6-2).

Syrian golden hamsters (30 male and 30 female) were fed 0.1% (w/w) 3,3'-dichlorobenzidine in the diet (40% dihydrochloride, 60% free base) ad libitum for their lifetime (Saffiotti et al., 1967). A similar group of controls received an untreated diet. The average food intake was 60 g/hamster/week and this led to a calculated 3,3'-dichlorobenzidine dose of 60 mg 3,3'-dichlorobenzidine/hamster/week. 3,3'-Dichlorobenzidine administration failed to produce any significant carcinogenic effect or bladder pathology in the treated hamsters. In a follow-up study by the same group of investigators (Sellakumar et al., 1969) a similar number of hamsters was fed a diet containing 0.3% (w/w) 3,3'-dichlorobenzidine. At this dietary level, 3,3'-dichlorobenzidine was reported to have induced four transitional cell bladder carcinomas, some liver-cell and cholangiomatous tumors and diffuse chronic intrahepatic obstructing cholangitis. This study

TABLE 6-2

Incidence of Tumors in Chr-CD Rats Treated with 3,3-Dichlorobenzidine in the Diet[a]

Sex	Dose (ppm)	Duration of Treatment[b] (days)	Target Organ	Tumor Type	Tumor Incidence[c] (p value)[d]
M	1000	353	blood	granulocytic leukemia	9/44 (p<0.05)
			mammary	adenocarcinoma	7/44 (p<0.05)
			skin	Zymbal gland carcinoma	8/44 (p<0.05)
F	1000	349	mammary	adenocarcinoma	26/44 (p<0.05)
M	0	564	blood	granulocytic leukemia	2/44
			mammary	adenocarcinoma	0/44
			skin	Zymbal gland carcinoma	0/44
F	0	628	mammary	adenocarcinoma	3/44

QUALITY OF EVIDENCE

Strength of study: Compound was administered by a relevant route of exposure to a sufficient number of both sexes of one species for a sufficient duration.

Weakness of study: Only one dose level was used.

Overall adequacy: Adequate

[a]Source: Stula et al., 1975

[b]Duration of treatment equals the duration of the study

[c]Tumor incidence expressed as number of animals with tumors/number of animals in group

[d]Chi-square method

(Sellakumar et al., 1969) was available only as an abstract and there were very few details given regarding length of study, actual tumor incidences and statistical significance.

Twenty-six male ICR/JCL mice were fed diets containing 0.1% 3,3'-dichlorobenzidine (purity unspecified) for up to 12 months (Osanai, 1976). Eight of the 3,3'-dichlorobenzidine-treated mice were sacrificed at 6 months and the remaining 18 at 12 months. Of 39 control mice fed the standard diet, 5 were sacrificed at 6 months, 21 were sacrificed at 12 months and 13 were sacrificed at 18 months. The incidence of hepatomas in 3,3'-dichlorobenzidine-treated mice was 100% at both sacrifice times (Table 6-3). Control mice killed at 6, 12 and 18 months had hepatoma incidences (and mean numbers of tumors) of 0, 9.5% (two hepatomas/mouse) and 38.5% (five hepatomas/mouse), respectively.

The data base regarding carcinogenicity of 3,3'-dichlorobenzidine in humans is limited. The available epidemiological studies are summarized as follows.

3,3'-dichlorobenzidine is suspected of being a bladder carcinogen in humans based on its structural resemblance to the known human bladder carcinogen, benzidine. Based on results of an epidemiological study of 207 workers exposed to 3,3'-dichlorobenzidine during the manufacture of dyes at the Allied Chemical Corp., Haledon, NJ (Gerarde and Gerarde, 1974), the authors concluded that 3,3'-dichlorobenzidine does not cause bladder cancer in humans. Individuals included in the study were those who had worked with or come in contact with 3,3'-dichlorobenzidine between 1938 and 1975, and their occupational and medical histories were examined. These were complete medical histories or follow-up reports on 175 workers (163 living and 12

TABLE 6-3

Incidence of Hepatomas in Male ICR/JCL Mice Fed 0.1% 3,3-Dichlorobenzidine in the Diet[a]

Dose	Duration of Treatment[b] (months)	Tumor Incidence[c] (p value)
0	6	0/5
0	12	2/21
0	18	5/13
0.1%	6	8/8 (p=0.0008)
0.1%	12	18/18 ($p=3\times10^{-9}$)

QUALITY OF EVIDENCE

Strengths of study: Natural route of exposure

Weakness of study: Small number of animals/group; only males tested; purity of compound not specified; only one dose tested; high spontaneous rate of tumor development in control.

Overall adequacy: Limited

[a]Source: Osanai, 1976

[b]Duration of the study equals the duration of treatment

[c]Fisher Exact test performed at SRC

deceased) having exposure to 3,3'-dichlorobenzidine ranging from 1 month to 24 years with the majority exposed for <15 years.

A similar retrospective epidemiological study was conducted of workers handling 3,3'-dichlorobenzidine in a plant in Britain (MacIntyre, 1975). There were no cases of bladder cancer found in a population of 225 workers exposed to 3,3'-dichlorobenzidine over a period of 30 years (with the majority exposed for less than 16 years).

A problem that arises when studying occupational exposure to 3,3'-dichlorobenzidine is that often workers who handle 3,3'-dichlorobenzidine are also exposed simultaneously to other known human carcinogens such as benzidine. Gadian (1975) studied workers at the Clayton Aniline Company who had worked with 3,3'-dichlorobenzidine or benzidine between 1953 and 1973. The incidence of urinary tract tumors in workers exposed to 3,3'-dichlorobenzidine and benzidine was 3/14 (2 carcinomas of bladder and 1 papilloma of bladder), whereas no bladder tumors were found in 35 workers exposed to 3,3'-dichlorobenzidine only. Gadian (1975) concluded that although the number of workers was small in this study, the findings did suggest that 3,3'-dichlorobenzidine does not cause bladder tumors in humans.

The three epidemiological studies (Gerarde and Gerarde, 1974; Gadian, 1975; MacIntyre, 1975) discussed above have been criticized by IARC (1982) because of a number of deficiencies. All of these studies examined relatively small cohorts of workers (the largest cohort consisted of 225 workers) and they all had limited statistical power to detect increases in bladder cancer. Most of the workers included in these three studies were exposed to 3,3'-dichlorobenzidine for <20 years and follow-up of exposed workers in the study by Gerarde and Gerarde (1974) was <85% complete.

Because of the high survival rate and long latency, increased incidences of bladder tumor may not be apparent if cohorts are not followed for a long period of time or if only mortality of the cohorts is analyzed.

An explanation of the finding that 3,3'-dichlorobenzidine does not cause bladder tumors in humans but apparently is capable of producing a variety of tumors in animals was proposed by Parkes and Evans (1984), who suggested that the levels of 3,3'-dichlorobenzidine to which workers are exposed are sufficiently low, compared with the experimental doses used in animals, so that no carcinogenic effect is observed.

6.2.3. Other Relevant Information. The carcinogenicity of 3,3'-dichlorobenzidine following subcutaneous administration in rats was studied by Pliss (1959). Twenty-five female and 36 male rats received weekly subcutaneous injections of an 8.8% suspension of 3,3'-dichlorobenzidine in glycerol at a dose of 120 mg 3,3'-dichlorobenzidine/rat for the first 5 months of the study. Because of toxic effects the dose was reduced to 20 mg/rat beginning on the 6th month. The total 3,3'-dichlorobenzidine dose over the entire period of the study (10-11 months) was ~1.62 g/rat. The animals were observed for life. Survival was 40 rats at 6 months, 35 rats at the time of appearance of the first tumor (7 months) and 23 rats at 12 months. Control animals (130 rats) were injected with either octadecylamine or methylstearylamine and were observed for 23 months. Of the rats surviving to the time of the first tumor, 26 (74.3%) had tumors at different sites. Ten rats had tumors of the Zymbal gland, five had skin tumors, six had mammary gland tumors, seven had local subcutaneous sarcomas, two had remote tumors of the connective tissue, two had haematopoietic system tumors and one had a salivary gland tumor. No tumors were observed in control animals.

In another study by Pliss (1963), an unspecified number of rats received subcutaneous injections of 3,3'-dichlorobenzidine (15-60 mg/rat) at unspeci-

fied intervals for 10-13 months. Fifty control rats were injected with the vehicle alone (sunflower seed oil or glycerol) or left untreated. Of the 3,3'-dichlorobenzidine-treated rats 74% developed tumors, with skin, sebaceous and mammary gland tumors being observed most frequently. One tumor was observed in control rats.

Both additive and synergistic tumorigenic effects were noted in rats given simultaneous or sequential administration of 3,3'-dichlorobenzidine, BNN, FANFT and 2-AAF (Ito et al., 1983). Simultaneous administration of 3,3'-dichlorobenzidine and BBN, or 3,3'-dichlorobenzidine, BBN and 2-AAF in the drinking water and diet of rats resulted in a significant synergistic effect of these chemicals on urinary bladder carcinogenesis over that seen when the chemicals were administered singly. Significant additive effects on urinary bladder carcinogenesis were seen when BBN, FANFT, 2AAF and 3,3'-dichlorobenzidine were given sequentially to rats in the diet or in drinking water (Ito et al., 1983).

3,3'-Dichlorobenzidine has also been demonstrated to function as a transplacental carcinogen (Golub et al., 1975). A group of 24 BALB/c mice (11 female and 13 male) was treated with five subcutaneous injections of 3,3'-dichlorobenzidine during the last week of pregnancy (2 mg/injection, total dose of 10 mg/mouse). A control group of 30 mice was treated with vehicle only (0.1 mℓ sunflower oil). All experimental animals, including treated animals and offspring of treated animals, were observed over a lifetime. The incidence of tumors in the offspring of 3,3'-dichlorobenzidine-treated mice was 13/24 compared with a tumor incidence of 6/30 in the offspring of controls. The incidence of lymphoid leukemias was significantly different between offspring of 3,3'-dichlorobenzidine-treated mice (incidence of 7/24) and control offspring (incidence of 0/30).

An *in vitro* method using degranulation of microsomes has been shown to successfully predict the carcinogenic nature of a number of compounds, including 3,3'-dichlorobenzidine (Jagota and Dani, 1985; Gupta and Dani, 1986). In this assay, rat liver microsomes are prepared by using a method that uses sedimentation at low G force (10,000 g). Microsomes prepared in this manner contain a large number of ribosomes/unit area, and incubation of these microsomes with known carcinogens has been shown to result in significant degranulation of the microsome. Incubation of the microsomes with noncarcinogens does not result in a high degree of degranulation. Of a number of carcinogens tested, 3,3'-dichlorobenzidine was demonstrated to cause a high percentage of microsomal degranulation.

3,3'-Dichlorobenzidine has also been demonstrated to be capable of producing cell transformation *in vitro* in high-passage rat embryo cell cultures (Freeman et al., 1973). 3,3'-Dichlorobenzidine was active in this assay at a concentration of 5.0 µg/mℓ but not at 1.0 µg/mℓ.

6.3. MUTAGENICITY

3,3'-Dichlorobenzidine has been tested for mutagenicity using various strains of *Salmonella typhimurium* (Garner, 1975; Lazear and Louie, 1977; Anderson and Styles, 1978; Reid et al., 1984; Iba, 1986; Vithayathil et al., 1983; Savard and Josephy, 1986; Prival et al., 1984; DeFrance et al., 1986; Commoner, 1976; Gentile et al., 1985). The results shown in Table 6-4 indicate that 3,3'-dichlorobenzidine acts as a mutagen towards *Salmonella typhimurium*, both in the presence and absence of metabolic activation (i.e., various liver S-9 preparations). The direct mutagenic activity of 3,3'-dichlorobenzidine has, however, been demonstrated by several investigators (Garner, 1975; Lazear and Louie, 1977; DeFrance et al., 1986) to be increased anywhere from 3- to 50-fold by the addition of liver metabolizing

TABLE 6-4

Mutagenicity Testing of 3,3'-Dichlorobenzidine

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	Salmonella typhimurium TA1538	purified DCB	plate incorporation	50 or 100 µg/plate	±S-9 (rat liver)	+/+	Direct mutagenic activity of compound was increased 50-fold by addition of liver S-9 preparation	Garner, 1975
		technical grade DCB sulfate	plate incorporation	50 or 100 µg/plate	±S-9 (rat liver)	+/+		
Reverse mutation	S. typhimurium TA98	DCB/NR	plate incorporation	50 or 100 µg/plate	±S-9 (mouse liver)	+/+	Direct mutagenic activity of compound was increased by ~3- to 6-fold by addition of liver S-9 fraction	Lazear and Loule, 1977
		DCB-2HCl/ NR	plate incorporation	50 or 100 µg/plate	±S-9 (mouse liver)	+/+		
Reverse mutation	S. typhimurium TA1535, TA1538, TA98, TA100	NR/NR	plate incorporation	50-500 µg/plate	+S-9	+	NC	Anderson and Styles, 1978
Reverse mutation	S. typhimurium TA98, TA1538	DCB-2HCl/ >98%	plate incorporation	5-20 µg/plate	+S-9	+	NC	Reid et al., 1984
Reverse mutation	S. typhimurium TA98	NR/NR	plate incorporation	NR	+S-9	+	Pretreatment of rats with phenobarbital enhanced S-9-catalyzed mutagenicity of DCB by 2.3-fold	Iba, 1986
Reverse mutation	S. typhimurium TA98	NR/NR	plate incorporation	10 µg/plate	+S-9	+	S-9 prepared from Aroclor-induced rat liver	Vithoyathil et al., 1983
Reverse mutation	S. typhimurium TA98, TA98/ 1,8-DNP_6	NR/>99%	plate incorporation	1-300 nmol/plate	±S-9	+/+	S-9 prepared from Syrian golden hamsters	Savand and Josephy, 1986
Reverse mutation	S. typhimurium TA98	NR/NR	plate incorporation	1-10 µg/plate	+S-9	+	Liver S-9 preparation from phenobarbitone pretreated rats was more effective in converting DCB to mutagen then liver S-9 from Aroclor 1254 pretreated rats	Booth et al., 1980

TABLE 6-4 (cont.)

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	S. typhimurium TA98	DCB-2HCl/ NR	plate incorporation	10-30 nmol/plate	S-9 (rat liver)	+	NC	Prival et al., 1984
Reverse mutation	S. typhimurium TA98	NR/NR	plate incorporation	1-10 nmol/plate	±S-9 (hamster liver)	+/+	Direct mutagenic activity of compound was increased ~4-fold by addition of liver S-9 fraction	Defrance et al., 1986
Reverse mutation	S. typhimurium TA98	NR/NR	plate incorporation	0-200 µg/plate	+S-9 (hamster liver)	+	NC	Commoner, 1976
Reverse mutation	S. typhimurium TA98	NR/NR	plate incorporation	10 µg/plate	+S-9 (hamster liver)	+	Results were similar for S-9 obtained from livers of Shistosome-infested hamsters control hamsters	Gentile et al., 1985
	S. typhimurium TA1538	NR/NR	plate incorporation	0.5-10 µg/plate	+S-9 (from Aroclor 1254-induced rat liver)	+	Increasing concentrations of Aroclor 1254-induced rat liver S-9 decreased DCB mutagenicity	Gentile et al., 1985
SCE	Bloom syndrome B-lymphoblastoid cell line	NR/NR	in vitro cell culture	1.7×10^{-8} to 1.3×10^{-2} M	±S-9 (rat liver)	+/+	SCE/cell were greater in presence of S-9	Shiraishi, 1986
Unscheduled DNA synthesis	HeLa cells	NR/NR	in vitro cell culture	10^{-4} to 10^{-7} M	+S-9 (rat liver)	+	NC	Martin et al., 1978

NC = No comment; NR = not reported

enzymes. Iba (1986) reported that epoxidation of 3,3'-dichlorobenzidine to form an arene oxide may be involved in the activation of the compound.

The type of inducer of liver enzymes used before the preparation of S-9 also appears to be important when studying the mutagenicity of 3,3'-dichlorobenzidine. It has been shown that Aroclor-1254 is a relatively poor inducer of the enzyme(s) responsible for 3,3'-dichlorobenzidine activation (Garner, 1980; Booth et al., 1980) and in fact increasing concentrations of liver S-9 from Aroclor 1254-induced rats have been shown to actually decrease the mutagenicity of 3,3'-dichlorobenzidine (Gentile et al., 1985).

3,3'-Dichlorobenzidine has also been demonstrated to be active in an in vitro assay measuring sister chromatid exchange in a B-lymphoblastoid cell line (Shiraishi, 1986) and in an in vitro assay measuring unscheduled DNA synthesis in HeLa cells (Martin et al., 1978).

6.4. TERATOGENICITY

Shabad et al. (1972) studied the effects of 3,3'-dichlorobenzidine administration to pregnant mice (during last week of pregnancy) on the embryonic kidney. Pregnant Balb/c mice were treated by subcutaneous injection with 8-10 mg 3,3'-dichlorobenzidine in sunflower oil and then fragments of embryonic kidney were explanted in organ culture. Embryonic kidney cultures obtained from 3,3'-dichlorobenzidine-exposed mice were shown to have a longer survival time than control cultures and in addition there was an induction of hyperplasia of epithelial structures in the cultures obtained from 3,3'-dichlorobenzidine-exposed mice.

6.5. OTHER REPRODUCTIVE EFFECTS

Pertinent data regarding other reproductive effects of 3,3'-dichlorobenzidine were not located in the available literature cited in Appendix A.

6.6. SUMMARY

Pertinent data regarding the systemic toxicity of 3,3'-dichlorobenzidine following either subchronic or chronic inhalation exposure in humans and animals were not located in the available literature. Stula et al. (1978) indicated that beagle dogs exposed to 3,3'-dichlorobenzidine orally for periods up to 7.1 years showed signs of liver toxicity in the form of elevated SGPT activities. The oral LD_{50} of 3,3'-dichlorobenzidine in rats has been reported to be between ~4 and 7 g/kg bw (ACGIH, 1986).

Pertinent data regarding the carcinogenicity of 3,3'-dichlorobenzidine following inhalation exposure in humans and animals were not located in the available literature cited in Appendix A. Oral administration of 3,3'-dichlorobenzidine has been shown to produce a variety of tumors in rats (Pliss, 1959; Stula et al., 1975; Griswold et al., 1968), urinary bladder and liver tumors in dogs (Stula et al., 1978) and hamsters (Sellakumar et al., 1969), and hepatomas in mice (Osanai, 1976). Subcutaneous administration of 3,3'-dichlorobenzidine has also been demonstrated to produce tumors in rats (Pliss, 1959, 1963).

Both additive and synergistic tumorigenic effects were noted in rats following simultaneous or sequential administration of low levels of 3,3'-dichlorobenzidine along with low levels of other carcinogens (such as BBN, FANFT and 2-AAF) (Ito et al., 1983). 3,3'-dichlorobenzidine has also been demonstrated to function as a transplacental carcinogen in mice (Golub et al., 1975).

3,3'-Dichlorobenzidine has been suspected of being a human carcinogen because of its carcinogenic effects in animals and because it resembles the known human bladder carcinogen benzidine. Evidence from three epidemiological studies (Gerarde and Gerarde, 1974; MacIntyre, 1975; Gadian, 1975),

however, indicates that 3,3'-dichlorobenzidine is not a bladder carcinogen in humans.

3,3'-Dichlorobenzidine has been demonstrated to be mutagenic towards Salmonella typhimurium in the Ames assay, both with and without metabolic activation. Metabolic activation (i.e., presence of liver S-9) has, however, been demonstrated to increase the mutagenicity of 3,3'-dichlorobenzidine from 3- to 50-fold (Garner, 1975; Lazear and Louie, 1977; DeFrance et al., 1986). 3,3'-Dichlorobenzidine has also been demonstrated to be active in in vitro assays measuring unscheduled DNA synthesis (Martin et al., 1978) and sister chromatid exchange (Shiraishi, 1986).

Pertinent data regarding the tetratogenicity of 3,3'-dichlorobenzidine were not located in the available literature. One study (Shabad et al., 1972) demonstrated that transplacental exposure of mice to 3,3'-dichlorobenzidine had effects on the growth of embryonic kidney cells in culture.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

Because ACGIH (1987) lists 3,3'-dichlorobenzidine as a suspected human carcinogen, there is no TLV-TWA for 3,3'-dichlorobenzidine. 3,3'-Dichlorobenzidine is suspected of being a human carcinogen because of its structural resemblance to benzidine, the known human bladder carcinogen; and because 3,3'-dichlorobenzidine has been demonstrated to be carcinogenic in experimental animals (ACGIH, 1986).

7.2. AQUATIC

The data base for the aquatic toxicity of 3,3'-dichlorobenzidine is limited and guidelines and standards for the protection of aquatic organisms were not located in the available literature cited in Appendix A.

8. RISK ASSESSMENT

8.1. CARCINOGENICITY

8.1.1. Inhalation. Pertinent data regarding the carcinogenicity of 3,3'-dichlorobenzidine following inhalation exposure in either animals or humans were not located in the available literature cited in Appendix A.

8.1.2. Oral. In a study by Pliss (1959), 50 rats (15 female and 35 male) were fed 3,3'-dichlorobenzidine in a paste which provided a dose of 10-20 mg 3,3'-dichlorobenzidine/day. The dose was administered 6 days/week for 12 months (total dose of 4.5 g 3,3'-dichlorobenzidine/rat) and the rats were observed for a lifetime. Twenty-three of the rats developed a variety of tumors.

In rats fed 3,3'-dichlorobenzidine in the diet (1000 ppm) for 349-353 days (Stula et al., 1975), significantly increased incidences of granulocytic leukemias, mammary adenocarcinomas and Zymbal gland carcinomas were found in males compared with controls. In female rats treated with same regimen, the incidence of mammary adenocarcinomas was significantly increased as compared with controls.

No mammary tumors were found in female rats treated by gastric intubation with 10 doses of 3,3'-dichlorobenzidine over a 30-day period (total dose of 300 mg/rat), whereas the incidence of mammary tumors in DMBA-treated positive controls in the same experiment was 100% (Griswold et al., 1968).

Significantly increased incidences of bladder carcinomas and hepatocellular carcinomas, as compared with controls, were found in beagle dogs given a daily oral dose of 100 mg 3,3'-dichlorobenzidine in capsular form 3 times/ week for 6 weeks followed by 5 times/week for up to an additional 7 years (Stula et al., 1978).

No evidence of carcinogenicity was found in hamsters fed a diet containing 0.1% (w/w) 3,3'-dichlorobenzidine (60 mg/hamster/week) over the course of a lifetime (Saffiotti et al., 1967). Hamsters fed a diet containing 0.3% (w/w) 3,3'-dichlorobenzidine (dosing schedule unspecified), however, developed transitional cell bladder carcinomas and some liver-cell and cholangiomatous tumors (Sellakumar et al., 1969). Individual tumor incidences were not given.

In mice fed 3,3'-dichlorobenzidine in the diet (0.1% w/w) for up to 12 months (Osanai, 1976), the incidence of hepatomas was 100% at both sacrifice times (6 and 12 months), whereas control mice killed at 6, 12 and 18 months had hepatoma incidences of 0, 9.5 and 38.5%, respectively.

8.1.3. Other Routes. Pliss (1959) reported that in rats receiving weekly subcutaneous doses of 3,3'-dichlorobenzidine suspended in glycerol for 10-11 months (total dose of 1.62 g/rat), 74.3% had tumors at different sites. No tumors were observed in control rats.

In another study, Pliss (1963) found that 74% of rats receiving subcutaneous injections of 3,3'-dichlorobenzidine (15-60 mg/rat) at unspecified intervals for 10-13 months developed tumors, with skin, sebaceous and mammary gland tumors being observed most frequently. One tumor was observed in control rats.

Three epidemiological studies of workers occupationally exposed to low levels of 3,3'-dichlorobenzidine are inadequate for assessment of human carcinogenicity of 3,3'-dichlorobenzidine due to deficiencies such as: small cohorts, limited statistical power and short exposure periods (Gerarde and Gerarde, 1974; MacIntyre, 1975; Gadian, 1975).

8.1.4. Weight of Evidence. There is sufficient evidence from several studies (Pliss, 1959, 1963; Stula et al., 1975, 1978; Sellakumar et al., 1969; Osanai, 1976) that 3,3'-dichlorobenzidine functions as a carcinogen in

animals. Data from three epidemiological studies (Gerarde and Gerarde, 1974; MacIntyre, 1975; Gadian, 1975) have several methodological limitations (see Section 6.2.2.), hence there is inadequate human evidence for carcinogenicity. Because there is sufficient evidence in animals and inadequate evidence in humans, 3,3'-dichlorobenzidine is categorized in EPA Group B2 - probable human carcinogen (U.S. EPA, 1986b).

8.1.5. Quantitative Risk Estimates.

8.1.5.1. INHALATION -- Because pertinent data regarding the carcinogenicity of 3,3'-dichlorobenzidine following inhalation exposure were not located in the available literature, an inhalation q_1^* for 3,3'-dichlorobenzidine was not calculated.

8.1.5.2. ORAL -- The study chosen for the derivation of the oral q_1^* was that of Stula et al. (1975), in which female rats fed 3,3'-dichlorobenzidine in the diet (1000 ppm) over the course of a lifetime had a statistically significant increased incidence of mammary adenocarcinomas compared with controls. This incidence of mammary adenocarcinomas in female rats (59.1%) was larger than the incidences of granulocytic leukemia (20.5%), mammary adenocarcinoma (15.9%) and Zymbal gland carcinoma (18.2%) observed in male rats in the same study. The study by Stula et al. (1978) on the carcinogenicty of 3,3'-dichlorobenzidine in beagle dogs was not considered for derivation of a q_1^* because of the small number of dogs (six) used in the study. The study by Osanai (1976) of the effects of 3,3'-dichlorobenzidine exposure on the hepatoma incidence in mice was not considered for q_1^* development because of the small number of control animals and because the duration of exposure (6 or 12 months) was fairly short compared with the lifespan of the animal (2 years).

The oral q_1^* for 3,3'-dichlorobenzidine was calculated using the multistage model developed by Howe and Crump (1982). An unadjusted (animal)

q_1^* of 2.3×10^{-2} $(mg/kg/day)^{-1}$ was calculated using the incidences of mammary adenocarcinomas in female rats fed either 0 or 1000 ppm 3,3'-dichlorobenzidine in the diet over the course of a lifetime (range of days on test: 143-488). The exposure level of 1000 ppm 3,3'-dichlorobenzidine in the diet was converted to a dose of 50 mg/kg/day using a food factor of 0.05 for rats (U.S. EPA, 1986d). Multiplication of the unadjusted q_1^* by the cube root of the ratio of human body weight (70 kg) to rat body weight (0.35 kg) (U.S. EPA, 1986d) and by the cube of the ratio of the lifespan of the rat (730 days) (U.S. EPA, 1986d) to the average length of the experiment (349 days) results in a human q_1^* of 1.2 $(mg/kg/day)^{-1}$.

To derive the concentration of 3,3'-dichlorobenzidine in the drinking water associated with an increased lifetime risk of cancer at a risk level of 10^{-5}, the risk level was divided by the human q_1^* of 1.2 $(mg/kg/day)^{-1}$ to give a dose of 8.3×10^{-6} mg/kg/day. Multiplying this value by the human body weight (70 kg) and dividing by the amount of water consumed by an individual each day (2 ℓ) (U.S. EPA, 1986d) results in a concentration of 2.9×10^{-4} mg 3,3'-dichlorobenzidine/ℓ in the drinking water associated with a risk level of 10^{-5}. Concentrations of 3,3'-dichlorobenzidine in the drinking water associated with risk levels of 10^{-6} and 10^{-7} are 2.9×10^{-5} and 2.9×10^{-6} mg 3,3'-dichlorobenzidine/ℓ, respectively.

8.2. SYSTEMIC TOXICITY

8.2.1. Inhalation Exposures. Pertinent data regarding the systemic toxicity of 3,3'-dichlorobenzidine following subchronic or chronic inhalation exposure in either animals or humans were not located in the available literature cited in Appendix A. This precluded the derivation of inhalation RfDs. Furthermore, because there is sufficient evidence that 3,3'-dichlorobenzidine is a carcinogen, it is not appropriate to derive an RfD.

8.2.2. Oral Exposures. Dogs given an oral dose of 3,3'-dichlorobenzidine (100 mg/day) over an extended period of time (i.e., up to 7.1 years) showed signs of liver toxicity in the form of elevated SGPT activities (Stula et al., 1978). Because 3,3'-dichlorobenzidine has been demonstrated to be carcinogenic, subchronic and chronic oral RfDs were not derived.

9. REPORTABLE QUANTITIES

9.1. BASED ON SYSTEMIC TOXICITY

In a previous determination (U.S. EPA, 1986c), an RQ for 3,3'-dichlorobenzidine based on systemic toxicity was not derived because the data were considered insufficient to derive an RQ. Reevaluation of the data presented in the study of 3,3'-dichlorobenzidine carcinogenicity in beagle dogs (Stula et al., 1978) has led to the development of the RQ for 3,3'-dichlorobenzidine presented here. Since the study by Stula et al. (1978) is the only chronic study that gives toxicity information on 3,3'-dichlorobenzidine, it is the only study considered for RQ development. This study is discussed in Chapter 6 and is summarized in Table 9-1.

The only effect considered for RQ development is elevated SGPT activities in dogs (see Table 9-1), which occurred at an equivalent human dose of 3.8 mg/kg/day. Multiplication of this dose by the human body weight (70 kg) gives an MED of 266 mg/day, which corresponds to an RV_d of 1.9 (RV_d = -1.5 log MED + 5.5). An RV_e of 6 was assigned to the effect of elevated SGPT, which represents clinical evidence of hepatocellular necrosis. Multiplication of the RV_d (1.9) by the RV_e (6) gives a CS of 11. The RQ associated with this CS is 1000 (Tables 9-2 and 9-3).

The basis for the derivation of this RQ based on systemic toxicity of 3,3'-dichlorobenzidine is weak. The study by Stula et al. (1978) is the only study with chronic toxicity information on 3,3'-dichlorobenzidine, and only one dose level was used in this study. This dose level and the resulting effect of elevated SGPT activity therefore define a free-standing LOAEL. Because 3,3'-dichlorobenzidine has been demonstrated to be carcinogenic in animals, the more conservative RQ of 10 derived from the carcinogenicity data (see section 9.2) is recommended for the 3,3'-dichlorobenzidine.

TABLE 9-1

Oral Toxicity Summary for 3,3'-Dichlorobenzidine Using the Female Beagle[a]

No. at Start	Average Weight (kg)	Vehicle/ Physical State	Purity	Exposure	Transformed Animal Dose (mg/kg/day)	Equivalent Human Dose[b] (mg/kg/day)	Response
6	9.73[c]	gelatin capsule	100%	100 mg/day, 3 times/week for 6 weeks then 5 times/week for an additional 7 years (total 7.1 years) TWA = 71 mg/day[d]	7.3[e]	3.8	elevated SGPT activities

[a]Source: Stula et al., 1978

[b]Calculated by multiplying the animal transformed dose by the cube root of the ratio of the animal body weight to the human body weight (70 kg)

[c]Determined from body weight data provided in the study

[d]TWA dose calculated as follows:

$$\text{TWA} \quad \frac{(100 \text{ mg/day} \times 3 \text{ days/7 days} \times 6 \text{ weeks}) + (100 \text{ mg/day} \times 5 \text{ days/7 days} \times 364 \text{ weeks})}{370 \text{ weeks}} = 71 \text{ mg/day}$$

[e]71 mg/day ÷ 9.73 kg = 7.3 mg/kg/day

TABLE 9-2

Oral Composite Score for 3,3'-Dichlorobenzidine Using the Dog*

Animal Dose (mg/kg/day)	Chronic Human MED (mg/day)	RV_d	Effect	RV_e	CS	RQ
7.3	266	1.87	elevated SGPT activities	6	11.2	1000

*Source: Stula et al., 1978

TABLE 9-3

3,3'-Dichlorobenzidine

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose*:	266 mg/day
Effect:	elevated SGPT activities
Reference:	Stula et al., 1978
RV_d:	1.9
RV_e:	6
Composite Score:	11
RQ:	1000

*MED

9.2. BASED ON CARCINOGENICITY

There were no data available regarding the carcinogenicity of 3,3'-dichlorobenzidine following inhalation exposure. Studies considered for the development of an RQ based on carcinogenicity were presented in Section 6.2.2. and are summarized in Tables 6-1, 6-2 and 6-3. Six female dogs given 100 mg 3,3'-dichlorobenzidine/day, 3 times/week for the first 6 weeks and then 5 times/week for up to an additional 7 years (Stula et al., 1978) developed papillary transitional cell carcinomas of the urinary bladder and hepatocellular carcinomas. This study was not considered for RQ development because of the small numbers of animals used. In another study, male and female rats were fed 3,3'-dichlorobenzidine (1000 ppm) in the diet over the course of a lifetime (range of days on test: 143-488; average days on test: 349 days) (Stula et al., 1975). 3,3'-Dichlorobenzidine-exposed female rats had a statistically significant increased incidence of mammary adenocarcinomas compared with controls, and this study was chosen for q_1^* and RQ development. In another study considered for RQ development, mice were exposed to 3,3'-dichlorobenzidine in the diet (0.1% w/w) for 6 or 12 months (Osanai, 1976). The incidence of hepatomas in the 3,3'-dichlorobenzidine-treated mice was 100% in both groups. This study was not used for q_1^* or RQ development because of the small number of control animals (39 were used) and because the length of exposure (6 or 12 months) was relatively short compared to the lifetime of the animal.

3,3'-Dichlorobenzidine has been shown to be carcinogenic in a number of animal studies (see Section 6.2.), but three epidemiological studies (Gerarde and Gerarde, 1974; MacIntyre, 1975; Gadian, 1975) are inadequate for determining whether 3,3'-dichlorobenzidine causes cancer in humans. Therefore, 3,3'-dichlorobenzidine is categorized in EPA Group B2 - probable human carcinogen (U.S. EPA, 1986b).

The unadjusted $1/ED_{10}$ for 3,3'-dichlorobenzidine based on the study of Stula et al. (1975) is 0.15626 $(mg/kg/day)^{-1}$ and was derived using the multistage model developed by Howe and Crump (1982) (Table 9-4). Multiplication of this unadjusted $1/ED_{10}$ by the cube root of the ratio of human body weight (70 kg) to rat body weight (0.35 kg) and by the cube of the ratio of the lifespan of the animal (730 days) to the average length of the experiment (349 days) results in an F Factor for humans of 8.4 $(mg/kg/day)^{-1}$. This F Factor is the same as the one derived by U.S. EPA (1986c) and places 3,3'-dichlorobenzidine in Potency Group 2. Because 3,3'-dichlorobenzidine is categorized in EPA Group B2 and Potency Group 2 the compound has a MEDIUM hazard ranking under CERCLA. A medium hazard ranking is associated with an RQ of 10.

TABLE 9-4

Derivation of Potency Factor (F) for 3,3'-Dichlorobenzidine

Reference:	Stula et al., 1975	
Exposure Route:	oral, diet	
Species:	rat	
Strain:	CHR-CD	
Sex:	female	
Vehicle State:	3,3'-dichlorobenzidine mixed in standard diet containing 1% added corn oil	
Body Weight:	0.35 kg	
Duration of Treatment:	349 days	
Duration of Study:	349 days	
Lifespan of Animal:	730 days	
Target Organ:	mammary gland	
Tumor Type:	adenocarcinoma	
Experimental Dose or Exposures:	0 ppm	1000 ppm
Transformed Dose:	0 mg/kg/day	50 mg/kg/day
Tumor Incidences:	3/44	26/44
Unadjusted $1/ED_{10}$:	0.15626 $(mg/kg/day)^{-1}$	
Adjusted $1/ED_{10}$: (F factor)	8.36273 $(mg/kg/day)^{-1}$	

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APPENDIX A

LITERATURE SEARCHED

This HEED is based on data identified by computerized literature searches of the following:

CHEMLINE
TSCATS
CASR online (U.S. EPA Chemical Activities Status Report)
TOXLINE
TOXLIT
TOXLIT 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register
CAS ONLINE (Chemistry and Aquatic)
HSDB

These searches were conducted in October 1987, and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1986. Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 1987. TLVs: Threshold Limit Values for Chemical Substances in the Work Environment adopted by ACGIH with Intended Changes for 1987-1988. Cincinnati, OH. 114 p.

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Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY.

Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

Windholz, M., Ed. 1983. The Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

Battelle's Columbus Laboratories. 1971. Water Quality Criteria Data Book. Volume 3. Effects of Chemicals on Aquatic Life. Selected Data from the Literature through 1968. Prepared for the U.S. EPA under Contract No. 68-01-0007. Washington, DC.

Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. U.S. Dept. Interior, Fish and Wildlife Serv. Res. Publ. 137, Washington, DC.

McKee, J.E. and H.W. Wolf. 1963. Water Quality Criteria, 2nd ed. Prepared for the Resources Agency of California, State Water Quality Control Board. Publ. No. 3-A.

Pimental, D. 1971. Ecological Effects of Pesticides on Non-Target Species. Prepared for the U.S. EPA, Washington, DC. PB-269605.

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APPENDIX B

Cancer Data Sheet for Derivation of q_1^*

Compound: 3,3'-Dichlorobenzidine

Reference: Stula et al., 1975

Species/strain/sex: rat/Chr-CD/female

Route/vehicle: oral, diet

Length of exposure (le) = 349 days

Length of experiment (Le) = 349 days

Lifespan of animal (L) = 730 days

Body weight = 0.35 kg (assumed)

Tumor site and type: mammary adenorcarcinoma

Experimental Dose or Exposure	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested
1000 ppm x 0.05 (food factor)	50	26/44
	0	3/44

Unadjusted $q_1^* = 2.3270696 \times 10^{-2}$ $(mg/kg/day)^{-1}$

Human $q_1^* = 1.2337681$ $(mg/kg/day)^{-1}$

APPENDIX C

Summary Table for 3,3'-Dichlorobenzidine

	Species	Exposure	Effect	RfD or q_1^*	Reference
Inhalation Exposure					
Subchronic	NA	NA	NA	NA	NA
Chronic	NA	NA	NA	NA	NA
Carcinogenicity	ID	ID	ID	ID	ID
Oral Exposure					
Subchronic	NA	NA	NA	NA	NA
Chronic	NA	NA	NA	NA	NA
Carcinogenicity	rat	1000 ppm in diet	mammary adenocarcinomas	1.2 $(mg/kg/day)^{-1}$	Stula et al., 1975
REPORTABLE QUANTITIES					
Based on Chronic Toxicity:		1000			Stula et al., 1978
Based on Carcinogenicity:		10			Stula et al., 1975

NA = Not applicable; ID = Insufficient data