Toxicological Profile for

3,3′-DICHLOROBENZIDINE

Agency for Toxic Substances and Disease Registry
U.S. Public Health Service

TOXICOLOGICAL PROFILE FOR 3,3'-DICHLOROBENZIDINE

Prepared by:

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Under Subcontract to:

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Under Contract No. 205-88-0608

Prepared for:

Agency for Toxic Substances and Disease Registry
U.S. Public Health Service

In collaboration with:

U.S. Environmental Protection Agency

December 1989

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FOREWORD

The Superfund Amendments and Reauthorization Act of 1986 (Public Law 99-499) extended and amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law (also known as SARA) directed the Agency for Toxic Substances and Disease Registry (ATSDR) to prepare toxicological profiles for hazardous substances which are most commonly found at facilities on the CERCLA National Priorities List and which pose the most significant potential threat to human health, as determined by ATSDR and the Environmental Protection Agency (EPA). The lists of the most significant hazardous substances were published in the Federal Register on April 17, 1987, and on October 20, 1988.

Section 110 (3) of SARA directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. Each profile must include the following content:

> (A) An examination, summary and interpretation of available toxicological information and epidemiological evaluations on the hazardous substance in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects,
>
> (B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure which present a significant risk to human health of acute, subacute, or chronic health effects, and
>
> (C) Where appropriate, an identification of toxicological testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

This toxicological profile is prepared in accordance with guidelines developed by ATSDR and EPA. The original guidelines were published in the Federal Register on April 17, 1987. Each profile will be revised and republished as necessary, but no less often than every 3 years, as required by SARA.

The ATSDR toxicological profile is intended to characterize succinctly the toxicological and health effects information for the hazardous substance being described. Each profile identifies and reviews the key literature that

describes a hazardous substance's toxicological properties. Other literature is presented but described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

Each toxicological profile begins with a public health statement, which describes in nontechnical language a substance's relevant toxicological properties. Following the statement is material that presents levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health will be identified by ATSDR, the National Toxicology Program of the Public Health Service, and EPA. The focus of the profiles is on health and toxicological information; therefore, we have included this information in the front of the document.

The principal audiences for the toxicological profiles are health professionals at the federal, state, and local levels, interested private sector organizations and groups, and members of the public. We plan to revise these documents as additional data become available.

This profile reflects our assessment of all relevant toxicological testing and information that has been peer reviewed. It has been reviewed by scientists from ATSDR, EPA, the Centers for Disease Control, and the National Toxicology Program. It has also been reviewed by a panel of nongovernment peer reviewers and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

Walter R. Dowdle, Ph.D.
Acting Administrator
Agency for Toxic Substances and
Disease Registry

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1. PUBLIC HEALTH STATEMENT

1.1 WHAT IS DICHLOROBENZIDINE?

3,3'-Dichlorobenzidine (3,3'-DCB) salt, the major form in actual use, is a stable, grey to purple crystalline solid that does not evaporate. The compound does not occur naturally. It is manufactured for use in the production of dyes and pigments for printing inks, textiles, plastics and enamels, paint, leather, and rubber. 3,3'-DCB breaks down rapidly in water exposed to natural sunlight and in air, but lasts in soil for months. In air, it is estimated that half of the chemical can breakdown within 2 hours. In water exposed to natural sunlight, 3,3'-DCB is expected to breakdown rapidly with half being removed in approximately 90 seconds. More information can be found in Chapters 3 and 4.

1.2 HOW MIGHT I BE EXPOSED TO 3,3'-DCB?

3,3'-DCB does not occur naturally in air, soil, or water. Workers who manufacture, process and package 3,3'-DCB are the major population at risk from exposure to the chemical, which occurs primarily in the workplace as dihydrochloride salt. Possible exposure in the workplace involves both breathing of 3,3'-DCB suspended in air and skin contact with the chemical. Current levels of 3,3'-DCB in workplace air in the United States are not known. For the general population, exposure is most likely to occur by drinking water from wells contaminated with 3,3'-DCB from industrial discharge and waste disposal sites. Also, exposure may occur by eating soil contaminated with 3,3'-DCB. Available information indicates that the levels of 3,3'-DCB in ground water samples are generally very low. However, areas in the vicinity of discharge from dye-manufacturing and other industrial plants may be higher. 3,3'-DCB has been found in ground and surface water at 1% of hazardous waste sites and in soil at about 4% of over 500 sites. The chemical has no agricultural or food chemical uses; so exposure to 3,3'-DCB by eating contaminated food is not likely, except for possible exposure from eating fish which could possibly store 3,3'-DCB in their body fat. More information can be found in Chapter 5.

1.3 HOW CAN 3,3'-DCB ENTER AND LEAVE MY BODY?

In the workplace, 3,3'-DCB may possibly enter workers' bodies by breathing 3,3'-DCB contaminated dust and through skin contact. For the general population, the most likely route is by drinking contaminated water. 3,3'-DCB can also enter the body through contact with soil

containing the chemical. When 3,3'-DCB does enter the body very little of it leaves the body unchanged. Most of it (over 90%) is changed to related chemical substances, called metabolites which leave the body mainly in feces and to a lesser extent in urine within 72 hours after exposure. More information can be found in Chapter 2.

1.4 HOW CAN 3,3'-DCB AFFECT MY HEALTH?

Workers exposed to the salt form of 3,3'-DCB complained of sore throat, respiratory infections and stomach upset. However, it is not known if 3,3'-DCB salt causes these health effects because the workers may also have been exposed at the same time to other chemicals.

Death has occurred in experimental animals after eating, for brief periods of time, very high levels of DCB mixed in their food. In studies in which pregnant mice were exposed to the chemical, the kidneys of their offspring did not develop properly. The effects of 3,3'-DCB on the growth of children of women exposed to the chemical while pregnant have not been studied. Long-term exposure of experimental animals to moderate levels of 3,3'-DCB mixed with food can cause mild injury to the liver.

The major concern is that 3,3'-DCB may cause cancer in humans. Studies show 3,3'-DCB causes cancer of the liver, skin, mammary gland, bladder and blood forming tissues (leukemia) and other sites when eaten with food by experimental animals. The ability of 3,3'-DCB to cause cancer in humans has not been established; however, in view of available animal data, 3,3'-DCB should be thought of as a probable cancer-producing substance in humans. More information can be found in Chapter 2.

1.5 IS THERE A MEDICAL TEST TO DETERMINE IF I HAVE BEEN EXPOSED TO 3,3'-DCB?

Exposure to 3,3'-DCB can be determined by finding the chemical or its metabolites in urine. The test is not commonly available to the general population, but is available to workers, who may be exposed to the chemical at potentially hazardous levels in the workplace. The test is accurate and provides evidence that exposure has occurred. However, since 3,3'-DCB does not remain in the body, the test must be performed very soon after the possible exposure. Further, measured urine and tissue levels of 3,3'-DCB or its metabolites do not predict adverse health effects in man. More information can be found in Chapter 6.

1. PUBLIC HEALTH STATEMENT

1.6 WHAT LEVELS OF EXPOSURE HAVE RESULTED IN HARMFUL EFFECTS?

Tables 1-1 and 1-3 show that no information is available on harmful human health effects that result from breathing, eating or drinking food or water containing specific levels of the chemical. 3,3'-DCB has a mild odor, but no information was found about levels at which the chemical is first smelled.

The relationship between oral exposure to 3,3'-DCB and known health effects in animals is shown in Table 1-4. No information was found on health effects in animals from breathing 3,3'-DCB for brief or long-term exposure periods (Table 1-2).

1.7 WHAT RECOMMENDATION HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The U.S. Environmental Protection Agency (EPA) considers 3,3'-DCB to be a probable human carcinogen, and has placed several limits on the chemical in the environment in order to protect human health. Under the Clean Water Act (1977), EPA controls discharges of 3,3'-DCB to industrial wastewaters. The agency has listed 3,3'-DCB as a hazardous waste and requires that any spill of one pound or more be reported to the National Response Center.

The Food and Drug Administration (FDA) has classified 3,3'-DCB as a cancer causing substance (carcinogen). Federal law does not allow the use of any substance in food, food additives, coloring or drugs which has been found by appropriate test to cause cancer.

The Occupational Safety and Health Administration (OSHA) classifies 3,3'-DCB as a cancer-suspect agent and controls 3,3'-DCB at the workplace by making strict requirements to reduce 3,3'-DCB concentrations in workplace air and protect the health of workers. These include personal protective equipment, training, labeling, and posting and engineering controls. OSHA also requires that initial medical screening and regular medical examinations be made available to any employee who is exposed to 3,3'-DCB at potentially hazardous levels.

The National Institute of Occupational Safety and Health (NIOSH) considers 3,3'-DCB a suspect human carcinogen and recommends workplace practices and controls to reduce exposures to the lowest possible limit.

1. PUBLIC HEALTH STATEMENT

TABLE 1-1. Human Health Effects from Breathing 3,3'-DCB*

Short-term Exposure (less than or equal to 14 days)		
Levels in Air (ppm)	Duration of Exposure	Description of Effects
		The health effects resulting from short-term exposure of humans to air containing specific levels of 3,3'-DCB are not known.
Long-term Exposure (greater than 14 days)		
Levels in Air (ppm)	Duration of Exposure	Description of Effects
		The health effects resulting from long-term exposure of humans to air containing specific levels of 3,3'-DCB are not known.

*See Section 1.2 for a discussion of exposures encountered in daily life.

TABLE 1-2. Animal Health Effects from Breathing 3,3'-DCB

Short-term Exposure (less than or equal to 14 days)		
Levels in Air (ppm)	Duration of Exposure	Description of Effects
		The health effects resulting from short-term exposure of animals to air containing specific levels of 3,3'-DCB are not known.
Long-term Exposure (greater than 14 days)		
Levels in Air (ppm)	Duration of Exposure	Description of Effects
		The health effects resulting from long-term exposure of animals to air containing specific levels of 3,3'-DCB are not known.

TABLE 1-3. Human Health Effects from Eating or Drinking 3,3'-DCB*

Short-term Exposure (less than or equal to 14 days)		
Levels in Food (ppm)	Duration of Exposure	Description of Effects
		The health effects resulting from short-term exposure of humans to food containing specific levels of 3,3'-DCB are not known.
Levels in Water (ppm)		The health effects resulting from short-term exposure of humans to water containing specific levels of 3,3'-DCB are not known.
Long-term Exposure (greater than 14 days)		
Levels in Food (ppm)	Duration of Exposure	Description of Effects
		The health effects resulting from long-term exposure of humans to food containing specific levels of 3,3'-DCB are not known.
Levels in Water (ppm)		The health effects resulting from long-term exposure of humans to water containing specific levels of 3,3'-DCB are not known.

*See Section 1.2 for a discussion of exposures encountered in daily life.

TABLE 1-4. Animal Health Effects from Eating or Drinking 3,3'-DCB

Short-term Exposure (less than or equal to 14 days)		
Levels in Food (ppm)	Duration of Exposure	Description of Effects*
76,000	Not Reported	Death in about half the exposed rats.
Levels in Water (ppm)		The health effects resulting from short-term exposure of animals to water containing specific levels of 3,3'-DCB are not known.
Long-term Exposure (greater than 14 days)		
Levels in Food (ppm)	Duration of Exposure	Description of Effects*
320	3.5 yr	Liver injury and convulsions in dogs.
Levels in Water (ppm)		The health effects resulting from long-term exposure of animals to water containing specific levels of 3,3'-DCB are not known.

*These effects are listed at the lowest level at which they were first observed. They may also be seen at higher levels.

1.8 WHERE CAN I GET MORE INFORMATION?

If you have further questions or concerns, please contact your State Health or Environmental Department or:

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road, E-29
Atlanta, Georgia 30333

2. HEALTH EFFECTS

2.1 INTRODUCTION

This chapter contains descriptions and evaluations of studies and interpretation of data on the health effects associated with exposure to 3,3'-DCB. Its purpose is to present levels of significant exposure for 3,3'-DCB based on toxicological studies, epidemiological investigations, and environmental exposure data. This information is presented to provide public health officials, physicians, toxicologists, and other interested individuals and groups with (1) an overall perspective of the toxicology of 3,3'-DCB and (2) a depiction of significant exposure levels associated with various adverse health effects.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the data in this section are organized first by route of exposure--inhalation, oral, and dermal--and then by health effect--death, systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods--acute, intermediate, and chronic.

Levels of significant exposure for each exposure route and duration (for which data exist) are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear, determine whether or not the intensity of the effects varies with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown on the tables and graphs may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons or with the identification of persons with the potential to develop such disease may be interested in levels of exposure associated with "serious effects". Public health officials and project managers concerned with response actions at Superfund sites may want information on levels of exposure associated with more subtle effects in

humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAEL) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels, MRLs) are of interest to health professionals and citizens alike.

For certain chemicals, levels of exposure associated with carcinogenic effects may be indicated in the figures. These levels reflect the actual doses associated with the tumor incidences reported in the studies cited. Because cancer effects could occur at lower exposure levels, the figures also show estimated excess risks, ranging from a risk of one in 10,000 to one in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made, where data were believed reliable, for the most sensitive noncancer end point for each exposure duration. MRLs include adjustments to reflect human variability and, where appropriate, the uncertainty of extrapolating from laboratory animal data to humans. Although methods have been established to derive these levels (Barnes et al. 1987; EPA 1980a), uncertainties are associated with the techniques.

2.2.1 Inhalation Exposure

3,3'-DCB is not a volatile chemical. It exists in air attached to dust particles or bound to particulate matter. The absorption of 3,3'-DCB from such respirable particles depends in part on the size of the particle. Large particles tend to deposit in the upper airways and are subsequently cleared by ciliary action with little absorption across lung tissues. Smaller particles can penetrate more deeply into the respiratory tree where 3,3'-DCB absorption may be significant.

2.2.1.1 Death

No studies were located regarding lethal effects in humans or animals after inhalation exposure to 3,3'-DCB. No fatalities were observed in rats observed for 14 days following exposure for one hour to an atmosphere containing an unspecified concentration of DCB dihydrochloride dust (Gerade and Gerade 1974).

2.2.1.2 Systemic Effects

Respiratory. Gerarde and Gerarde (1974) listed upper respiratory infection and sore throat among several principal reasons for frequent visits to a company's medical clinic by workers handling 3,3'-DCB dihydrochloride. It is possible that these effects were due to

inhalation of 3,3'-DCB dihydrochloride salt although the results are not conclusive. No adverse health effects were observed in the respiratory system in rats exposed by inhalation to DCB free base (25 mg/m^3) two hours per day for seven days (Gerarde and Gerarde 1974). In another study, ten rats were exposed to an unspecified dose of DCB dihydrochloride dust particles for one hour and then observed for 14 days. Slight to moderate pulmonary congestion and one pulmonary abscess were observed upon autopsy (Gerarde and Gerarde 1974). Irritant effects of HCl from the chemical could have contributed to the observed effects in this study.

Gastrointestinal. Gastrointestinal upset was one of the frequent symptoms reported by employees who worked with DCB dihydrochloride (Gerarde and Gerarde 1974). There is no conclusive evidence that the gastrointestinal effects, or other symptoms reported by employees, resulted from inhalation of 3,3'-DCB dihydrochloride salt. No studies were located regarding gastrointestinal effects in animals following inhalation exposure to 3,3'-DCB.

Other Systemic Effects. No studies were located regarding cardiovascular, musculoskeletal, hepatic, renal or dermal/ocular effects in humans or animals after inhalation exposure to 3,3'-DCB.

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

2.2.1.3 Immunological Effects

2.2.1.4 Neurological Effects

2.2.1.5 Developmental Effects

2.2.1.6 Reproductive Effects

2.2.1.7 Genetic Effects

2.2.1.8 Cancer

2.2.2 Oral Exposure

Indirect gastrointestinal tract exposure may occur from breathing contaminated airborne dust in the workplace. The deposition pattern of inhaled 3,3'-DCB would depend primarily on the mass median aerodynamic diameter (MMAD) of the particles. The mucociliary clearance mechanism

moves most particulates with a MMAD of 1 to 5 μm out of the lungs and into the gastrointestinal tract. Larger particles (> 5μm) impacting in the nasopharyngeal region would also be eventually ingested. Oral exposure may potentially occur in the general environment by drinking contaminated groundwater. Occupational exposure by oral route is not expected to be significant. Exposure through eating food is unlikely since 3,3'-DCB has never had an application as an agricultural or food chemical. However, fish have been reported to bioconcentrate 3,3'-DCB (Appleton and Sikka 1980) under experimental conditions, raising the potential for bioaccumulation and human exposure. Individuals, such as children, consuming contaminated soil are at risk. All of the available data on the effects of 3,3'-DCB following oral exposure are derived from studies in experimental animals. Table 2-1 and Figure 2-1 summarize available data.

2.2.2.1 Death

No studies were located regarding lethal effects in humans after oral exposure to 3,3'-DCB. The acute oral LD_{50} of DCB in rats has been estimated to be 7,100 mg/kg for the free base and 3,800 mg/kg for the dihydrochloride salt (Gerarde and Gerarde 1974). Given this high LD_{50}, acute lethality in man following oral exposure is very unlikely. The dose of 3,800 mg/kg/day has been converted to an equivalent concentration (76,000 ppm) in food for presentation in Table 1-4.

2.2.2.2 Systemic Effects

Respiratory Effects. No studies were located regarding respiratory effects in humans or animals after oral exposure to 3,3'-DCB.

Hepatic Effects. No studies were located regarding hepatic effects in humans following oral exposure to 3,3'-DCB. Limited animal evidence suggests that chronic oral exposure to 3,3'-DCB results in mild to moderate liver injury. Six female dogs exposed to 3,3'-DCB (~8 mg/kg/day) all had modestly elevated plasma glutamic-pyruvic transaminase (GPT) during the first three years of a seven-year treatment period (Stula et al. 1978). Thereafter, GPT levels returned to normal in three of the experimental animals, but remained elevated in two of the animals for the duration of the study. The significance of this effect is unclear in the absence of pronounced histopathologic effects. Further, enzyme levels may be altered by other conditions. One of six dogs, sacrificed after 42 months on the test, showed a marked fatty change in the liver. It should be noted that the study is limited

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TABLE 2-1. Levels of Significant Exposure to 3,3'-DCB - Oral Exposure

Graph Key	Species	Route	Exposure Duration/ Frequency	Syst. Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
ACUTE EXPOSURE								
Lethality								
1	rat	(G)	NR				3800 (LD50) 7100 (LD50)	Gerarde and Gerarde 1974
Systemic								
2	rat	(G)	1 dose	Hepatic		500 (UDS)		Ashby and Mohammed 1988
3	mouse	(G)	1 dose	Hematol		1000 (micronuclei formation)		Cihak and Vontorvoka 1987
CHRONIC EXPOSURE								
Systemic								
4	dog	(C)	3.5-7 yr 3-5x/wk	Hematol	8			Stula et al. 1978
5	dog	(C)	3.5-7 yr 3-5x/wk	Hepatic		8 (GPT levels increased)		Stula et al. 1978
Neurological								
6	dog	(C)	sacrificed 3.5 yr 3-5x/wk				8 (convulsions, neuronal degeneration)	Stula et al. 1978
Carcinogenic								
7	rat	(F)	118-488d (350avg)				50 (CEL- multi-tumor sites)	Stula et al. 1975
8	dog	(C)	6.6-7 yr 3-5x/wk				8 (CEL-bladder liver tumor)	Stula et al. 1978

Syst = systemic; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; mg = milligram; kg = kilogram; G = gavage; NR = not reported; LD50 = lethal dose, 50% mortality; UDS = unscheduled DNA synthesis; Hematol = hematological; C = capsule; yr = year; x = time; wk = week; GPT = glutamic-pyruvic transaminase; F = feed; d = day; CEL = cancer effect level; avg = average.

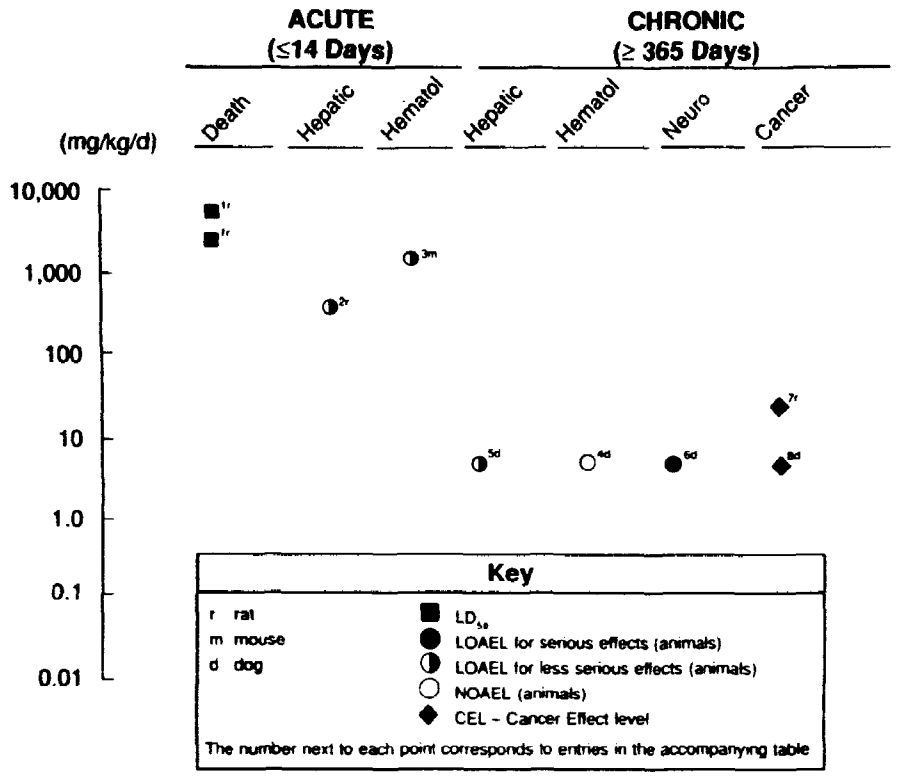

FIGURE 2-1. Levels of Significant Exposure to 3,3' DCB – Oral Exposure

by the use of one dose level, precluding dose-response evaluations, and the male dog was not evaluated. It is uncertain if these effects will also occur in humans orally exposed to 3,3'-DCB. The dose of 8 mg/kg/day has been converted to an equivalent concentration (320 ppm) in food for presentation in Table 1-4.

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to 3,3'-DCB. Hematological variables (erythrocyte count, hemoglobin concentration, hematocrit and leucocyte count) were found to be normal in dogs exposed to 3,3'-DCB for seven years (Stula et al. 1978). Hematological effects may not be sensitive indicators for 3,3'-DCB toxicity.

Other Systemic Effects. No studies were located regarding cardiovascular, gastrointestinal, musculoskeletal, renal or dermal/ocular effects in humans or animals after oral exposure to 3,3'-DCB.

2.2.2.3 Immunological Effects

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

2.2.2.4 Neurological Effects

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

Stula et al. (1978) reported that one out of the six dogs in a 3,3'-DCB carcinogenicity study exhibited convulsions after 21, 28, and 42 months of treatment with 8 mg/kg/day (320 ppm) over a period of 3.5 years (total 3,3'-DCB intake = 86 g). On autopsy at 42 months, slight neuronal degeneration was observed. In view of the fact that only one dog developed the lesion, causality cannot be inferred.

2.2.2.5 Developmental Effects

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

2.2.2.6 Reproductive Effects

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

2.2.2.7 Genetic Effects

No studies were located regarding genetic effects in humans after oral exposure to 3,3'-DCB; however, genotoxicity effects have been reported in animals. Cihak and Vontorvoka (1987) reported that a single dose of 3,3'-DCB (1000 mg/kg) administered to male and pregnant female mice induced micronuclei in polychromatic erythrocytes in the bone marrow of the males, in the liver of the fetuses, but not in bone marrow of the dams. A micronucleus test is performed to detect a chemical's ability to induce chromosomal aberrations. However, the relevance of micronuclei formation to human health is not known. The lack of effect of 3,3'-DCB on bone marrow micronuclei formation in the mothers is unclear but may be related to deficiencies in the metabolic activation of 3,3'-DCB peculiar to the pregnant state. The relative importance of this differential effect is reduced since the study did not evaluate nonpregnant females. In another study, Ashby and Mohammed (1988) reported an increase in unscheduled DNA synthesis (UDS) in cultured liver cells from male mice previously pretreated orally with single doses of 3,3'-DCB of 500 mg/kg or higher; no response was observed at a dose of 200 mg/kg or lower. The unscheduled DNA synthesis assay is used to measure the repair that follows DNA damage. However, the relevance of UDS to human health is not known. While results were positive in two in vivo assay systems, sufficient data are not available from more predictive indicator assays to adequately characterize the genotoxic potential for 3,3'-DCB in humans.

2.2.2.8 Cancer

There are no epidemiological studies linking cancer in humans to oral exposure to 3,3'-DCB. However, based on the findings of oral studies in animals 3,3'-DCB may be regarded as a probable carcinogen in humans. Stula et al. (1975) fed 50 male and 50 female ChRCD rats 1,000 ppm 3,3'-DCB in a standard diet for up to 16 months. Mammary adenocarcinoma (16% incidence), malignant lymphoma (14%), granulocytic leukemia (20%), carcinoma of the Zymbal gland (18%) in males, and mammary adenocarcinoma (59%) in females were observed in rats fed 3,3'-DCB in the diet (1,000 ppm or 50 mg/kg/day) for a total duration of 349 (females) to 353 (males) days, respectively (Stula et al. 1975). These tumors were either totally absent or occurred statistically less frequently in untreated controls (Stula et al. 1975). Both sexes were dosed orally and comprehensive histopathological evaluations were

performed. While only one dose level was used and the purity of the compound was not specified, these data are qualitatively significant. In female dogs fed approximately 8 mg/kg/day (320 ppm) orally in gelatin capsules over a period of 6.6 to 7.1 years (total 3,3'-DCB intake = 164 to 176 g), hepatocellular carcinomas and papillary transitional cell carcinomas of the urinary bladder were observed (Stula et al. 1978). These tumors were absent in untreated controls. It should be noted that a small number of dogs (6) were evaluated and only one sex and one dose were used. However, a sufficient number of animals survived to develop tumors. The results are qualitatively significant. Existing animal data show that 3,3'-DCB produces tumors in multiple organs in several animal species. Although these data were derived from studies judged to be limited in scope, they do suggest that 3,3'-DCB is a probable human carcinogen. The doses observed to cause cancer in experimental animals are presented in Table 2-1.

2.2.3 Dermal Exposure

Because of large particle size and increased usage of closed systems and protective clothing, dermal absorption may be expected to be minimal with the possible exception of conditions of high humidity and high temperature. Extremely limited data (1 rabbit, 1 exposure) were found regarding the effects of dermal exposure to 3,3'-DCB (Table 2-2 and Figure 2-2).

2.2.3.1 Death

No studies were located regarding lethal effects in humans after dermal exposure to 3,3'-DCB. The dermal LD50 for DCB (free base) for male and female New Zealand albino rats was reported to be greater than 8 g/kg bw (Gerarde and Gerarde 1974). The cause of death was not reported and the dose causing the effect was similar by a different route. Dermal exposure is not likely to cause death in humans.

2.2.3.2 Systemic Effects

Respiratory Effects. As mentioned in Section 2.2.1.2 on "Respiratory Effects of Inhalation Exposure to 3,3'-DCB", upper respiratory infection and sore throat were among the principal reasons for frequent visits to a company's medical clinic by workers who handled 3,3'-DCB (Gerarde and Gerarde 1974). No studies were located regarding respiratory effects in animals after dermal exposure to 3,3'-DCB.

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TABLE 2-2. Levels of Significant Exposure to 3,3'-DCB - Dermal

Graph Key	Species	Exposure Duration/ Frequency	Syst. Effect	NOAEL	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
ACUTE EXPOSURE							
1	rabbit	1 hr	OC			5 (Corneal opacity, irritation)	Gerarde and Gerarde 1974

Syst = systemic; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; hr = hour; oc = ocular.

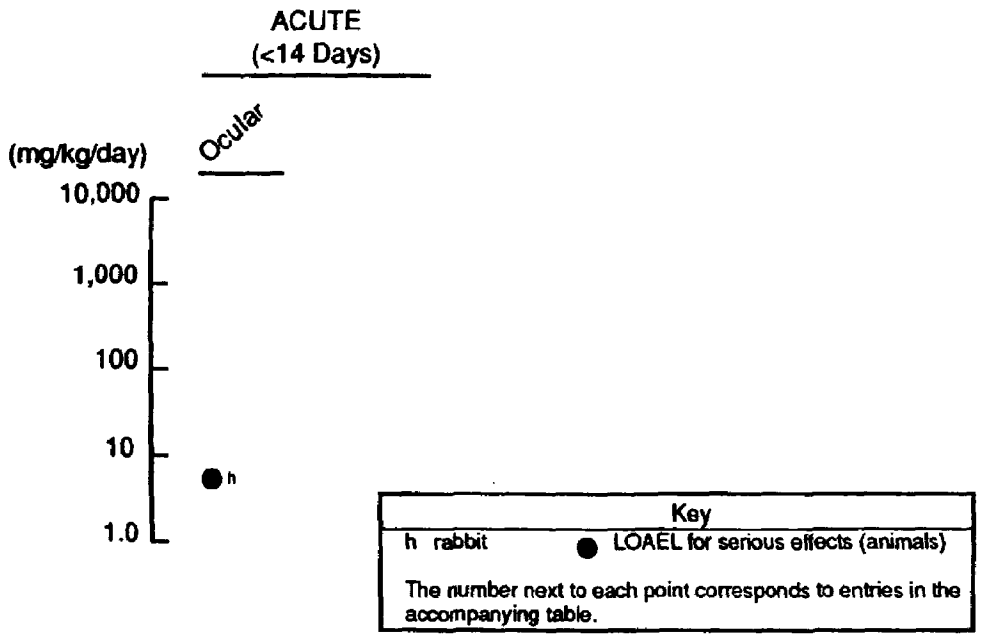


FIGURE 2-2. Levels of Significant Exposure to 3,3'-DCB – Dermal Exposure

2. HEALTH EFFECTS

Dermal/Ocular Effects. Dermatitis was cited as the only verified health problem encountered by workers in contact with the free base of 3,3'-DCB in a DCB manufacturing plant (Gerarde and Gerarde 1974). There was no discernable skin irritation when 3,3'-DCB dihydrochloride (at an unstipulated dose) was applied to the intact and abraded skin of rabbits (Gerarde and Gerarde 1974). Similarly, an aqueous suspension of DCB instilled intradermally into rats at a dose of 700 mg/kg did not produce adverse effects (Gerarde and Gerarde 1974). It is possible that solubility limitations contributed to the failure of DCB to cause skin irritation in the experimental animals examined. Alternatively, the duration of observation may not have been of sufficient length. The study did not specify the length of the observation periods. If, however, DCB indeed causes no skin irritation in experimental animals, dermatitis may be an effect of the chemical that is peculiar to humans.

No effects were reported in rabbits when 100 mg of DCB (free base) was placed in the conjunctival sac of the eye (Gerarde and Gerarde 1974). It should be noted that the authors did not report the duration of exposure or the vehicle used. However, 20 mg of DCB dihydrochloride (as 0.1 mL of 20% corn oil suspension) produced erythema, pus and corneal opacity, giving a 76% score in the Draize test within an hour when placed in the conjunctival sac of the eye of the rabbit (Gerarde and Gerarde 1974).

Other Systemic Effects. No studies were located regarding cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic or renal effects in either humans or animals after dermal exposure to 3,3'-DCB.

No studies were located regarding the following effects in humans or animals after dermal exposure to 3,3'-DCB.

2.2.3.3 Immunological Effects

2.2.3.4 Neurological Effects

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genetic Effects

2.2.3.8 Cancer

2. HEALTH EFFECTS

2.3 RELEVANCE TO PUBLIC HEALTH

Existing data are considered inadequate to derive human Minimal Risk Levels.

Systemic Effects. Dermatitis appears to be the only effect of 3,3'-DCB (free base) exposure for which evidence exists. However, a dose-response relationship can not be determined from available information. Gastrointestinal upset and upper respiratory tract infections have also been reported by workers, but 3,3'-DCB exposure conditions are unavailable. 3,3'-DCB has not been found to cause any of these effects in experimental animals. The extent to which these, or other effects of 3,3'-DCB exposure may occur predominantly in man is unknown.

Hepatic. No studies were located regarding hepatic effects in humans after exposure to 3,3'-DCB. Evidence suggestive of mild liver injury has been reported in one chronic animal experiment. Stula et al. (1978) reported that during a carcinogenicity study using six beagle dogs, all of the animals showed modest elevation in serum transaminase activity within one year of initiation of a regimen of oral DCB (total dose, 24.8 g). On continuation of the DCB regimen (500 mg/week), the serum transaminase levels returned to normal in three dogs. One dog, sacrificed in extremis after 42 months of 3,3'-DCB exposure was found to have a fatty liver. The interpretation of these data is complicated by the fact that on eventual sacrifice, all of the surviving DCB treated animals showed evidence of hepatocellular carcinoma, hepatic metastases or other focal cell alterations. It is thus possible that the modest elevations of serum transaminase activity reflects hyperplastic events rather than hepatic necrosis or other frank cellular injury. Further, since only one dose level was used in the study, dose response relationships were not established. Collectively, while the data is suggestive of mild hepatotoxicity, its significance for human toxicity is not clear.

Iba and colleagues (Iba 1987b; Iba and Lang 1988; Iba and Thomas 1988) administered 3,3'-DCB to rats (20 mg/kg i.p.) and observed a decrease in hepatic vitamin E levels *in vivo*, and an increase in lipid peroxidative activity in *in vitro* assays using hepatic microsomes isolated from DCB-treated rats. However, since the histology of the liver and other indices of hepatotoxicity were not examined, no conclusions can be drawn from these mechanistic studies as to the capacity of 3,3'-DCB to cause frank hepatocellular injury in rats.

2. HEALTH EFFECTS

Developmental Effects. No studies were located regarding developmental effects of 3,3'-DCB in humans following brief or long-term exposure. Abnormal growth was observed in kidneys explanted from fetuses of pregnant mice treated sc daily during the last week of pregnancy at an average daily dose of 257 mg/kg. This abnormal growth was not observed in vehicle-treated controls (Shabad et al. 1972). It should be noted that the authors did not provide data on maternal effects; therefore, these findings should be interpreted with caution. Similarly, in subcutaneous injection studies in BALB/C-mice, hyperplastic foci and hyperchromic glomeruli were observed in kidneys of offspring of dams administered 2 mg 3,3'-DCB throughout gestation. (Golub 1970). No data were reported on maternal effects. These observations suggest a potential for the chemical to act as a developmental toxicant in experimental animals.

Genotoxic Effects. Studies in several test systems show 3,3'-DCB to be genotoxic in vitro and in vivo (Tables 2-3 and 2-4) and suggest that this effect most likely mediates the carcinogenicity of the chemical. In vivo, micronuclei were induced in polychromatic erythrocytes of the liver of fetal mice exposed transplacentally to the chemical, and in liver cells of adult male mice treated orally with the chemical at a maximum tolerated dose reported to be 1000 mg/kg (Cihak and Vontorvoka 1987). A sex difference in the genotoxicity of the chemical is suggested as adult male mice, but not pregnant females developed erythrocyte micronuclei following 3,3'-DCB exposure. However, whether this differential effect extends to carcinogenic effects is unclear. Positive chromatid exchange findings in an in vitro test system provide supportive evidence for 3,3'-DCB-induced cytogenetic changes. Shiraishi (1986) reported 3,3'-DCB induced sister chromatid exchanges (SCEs) in all types of Bloom Syndrome (BS) B-lymphoblastoid cell lines. The induction of SCE was variable among the three types. Exposure of BS type II and type III cells to 3,3'-DCB (1.7×10^{-8} to 1.3×10^{-3}) caused an increase in SCEs (120 - 140/cell) over baseline levels (70/cell) at the highest concentration (1.3×10^{-3}). BS type II cells required metabolic activation, while BS type III cells were sensitive with and without activation. The frequency of SCEs in BS type I cells was lower than in II and III.

The genotoxic effect of 3,3'-DCB is further supported by positive responses in bacterial assays employing Salmonella tester strains TA1538 and TA98 in the absence of liver activating systems (Garner et al. 1975; Iba 1987a; Iba and Thomas 1988; Lang and Iba 1987; Lazear et al. 1979; Savard and Josephy 1986). This direct mutagenicity may be due to the metabolic activation of the chemical by enzymes endogenous to the

TABLE 2-3. Genotoxicity of 3,3'-DCB *In Vitro*

End Point	Test System	Activation System	Results: Without Activation	Results: With Activation	Reference
Gene mutation	*Salmonella typhimurium*	Rat S-9	+	+	Garner et al. 1975 Lazear et al. 1979 Savard and Josephy 1986 Iba 1987a Lang and Iba 1987
Unscheduled DNA synthesis	HELA cells	Rat S-9	-	+	Martin et al. 1978
Cell transformation	rat embryo cells	-	+	NA	Freeman et al. 1973
Cell transformation	hamster kidney cells	Rat S-9	+	-	Styles 1978
Sister chromatid exchange	Lymphoblastoid cells	Rat S-9	_	+	Shiraishi 1986

+ = positive result; - = negative result; NA = not available.

TABLE 2-4. Genotoxicity of 3,3'-DCB In Vivo

End Point	Species	Results	Reference
Micronucleus test	mice	+	Cihak and Vontorvoka 1987
Unscheduled DNA synthesis	rat	+	Ashby and Mohammed 1988

+ = positive result.

bacteria (Lang and Iba 1987), or the chemical may be a direct acting genotoxic agent. The bacterial mutagenicity is highly enhanced in the presence of liver activating systems indicating the importance of metabolism in the genotoxicity of the chemical.

Based on mutagenicity findings, 3,3'-DCB is an effective inducer of its own activation (Iba 1987a). The enhancing effect of 3,3'-DCB pretreatment on the in vitro liver activation of the chemical to mutagens has been associated with the induction of cytochrome P-450d (Iba and Thomas 1988). This action may result in the chemical enhancing its own genotoxicity and carcinogenicity. Iba and Sikka (1982) reported that 3,3'-DCB is a potent inducer of hepatic microsomal enzymic activities mediated by cytochrome-P-448. The authors noted that some of the toxic properties as well as the carcinogenicity of compounds such as the polycyclic aromatic hydrocarbons and the polyhalogenated aromatics may be related to their ability to induce cytochrome P-448 mediated monooxygenase activities. They therefore concluded that hepatocarcinogenicity of 3,3'-DCB may thus be due, at least in part, to the induction of hepatic cytochrome P-448.

Results of in vivo tests show 3,3'-DCB induced dose-dependent unscheduled DNA synthesis in the liver of male rats treated orally (Ashby and Mohammed 1988). In vitro evidence for the genotoxicity of 3,3'-DCB include the induction of unscheduled DNA synthesis in HeLa cells at a concentration range of 10^{-7} to 10^{-4}M (Martin et al. 1978), and transformation of high passage rat embryo cells infected with the Rauscher leukemia virus (Freeman et al. 1973). In the latter system, an effect was observed at $2x10^{-7}$M 3,3'-DCB but not at $4x10^{-8}$M. Also, 3,3'-DCB transformed BHK21 cells (hamster kidney cells) in vitro in the presence of metabolic activation (Styles 1978).

3,3'-DCB formed adducts with calf thymus DNA when incubated with rat liver S9 (Bratcher and Sikka 1982), or horseradish peroxidase (Tsuruta et al. 1985) in vitro. The relevance of DNA adduct formation to the genotoxicity and carcinogenicity of the chemical is not yet established.

Overall, there is convincing evidence that 3,3'-DCB is genotoxic in animals. Results were positive in two in vivo (UDS, and micronucleus formation) test systems. Supporting evidence was provided by one bacterial assay (Salmonella). However, these in vivo and in vitro tests are usually of limited predictive value in man. Therefore, the genotoxicity potential of 3,3'-DCB in man, although a possibility, remains uncertain.

2. HEALTH EFFECTS

Cancer. 3,3'-DCB may cause tumors in multiple organs in man as suggested by findings in experimental animals. Epidemiological studies of potential effects of occupational exposure to 3,3'-DCB have paid particular attention to bladder tumors. To date, none of these studies have found either bladder tumors or excess tumors at other sites that are clearly attributable to exposure to 3,3'-DCB. Despite concerns of the chemical's ability to induce bladder tumors, this form of cancer has not been satisfactorily investigated in occupationally exposed workers.

It has been speculated (IARC 1982a) that 3,3'-DCB may have contributed to the incidence of bladder cancer attributed to benzidine in dye industry workers who handled both benzidine and 3,3'-DCB (Gadian 1975). However, no bladder tumors were produced in another group of workers who handled 3,3'-DCB exclusively within the same plant (Gadian 1975). The author reported a total exposure time of 68,505 hours, equivalent to nearly 140 full-time working years.

Cystodiagnostic tests produced no indication of tumors of the bladder in an epidemiological study of 225 workers who had been exposed for a total of less than 16 years to 3,3'-DCB (MacIntyre 1975).

In a retrospective epidemiological study, no bladder tumors were observed in a cohort of 207 workers, most of whom had been exposed to 3,3'-DCB for up to 15 years (Gerarde and Gerarde 1974).

It should be pointed out that observations in two of the three studies were made in workers who were exposed to 3,3'-DCB for less than 20 years. Since the average latency period for chemically induced bladder cancer in man is 18 years, an adequate latency period for 3,3'-DCB-induced tumors may not have elapsed. Also, the number of workers examined in the above three studies was relatively small, thus limiting the statistical power to detect significant (two-fold) increase in bladder cancer mortality (incidence).

3,3'-DCB has been found to cause neoplasia in a variety of target organs in several animal species. The chemical produces hepatocellular carcinomas and urinary bladder carcinomas in dogs and hamsters. Liver cell tumors were demonstrated in 3,3'-DCB exposed mice. In rats, mammary gland tumors, Zymbal gland tumors and leukemias were attributable to 3,3'-DCB exposure. (Pliss 1963; Stula et al. 1975, 1978). While concordance between tumor sites in experimental animals and man cannot be assumed, the occurrence of multiple target organs in experimental animals should be regarded as evidence for the potential carcinogenicity of 3,3'-DCB to man.

In subcutaneous injection studies, induction of tumors in progeny of BALB/C-mice administered 2 mg 3,3'-DCB during the last week of pregnancy suggest that the chemical may be a transplacental carcinogen (Golub et al. 1975). There was an increased incidence of lymphatic leukemias (7/24 or 29%), lung adenomas (5/24 or 20%) and adenocarcinomas of the mammary gland (4/24 or 17%) in the treated group. Lung tumors (3/30 or 10%) and mammary gland tumors (3/30 or 10%) were observed in untreated controls.

The route of 3,3'-DCB exposure may be important in the carcinogenicity of the chemical as suggested by the results of animal studies. Following subcutaneous administration in rats, the compound was found to cause tumors of the skin and intestines. These sites were in addition to tumors of the mammary gland, hematopoietic organs and Zymbal gland which predominate following oral exposure (Pliss 1963).

The results of industrial plant surveys suggest that the dermal route is a minor source for exposure to 3,3'-DCB. Studies have not been located which investigate the carcinogenic potential of 3,3'-DCB following dermal exposure in laboratory animals.

Overall, there is convincing evidence that 3,3'-DCB is genotoxic and carcinogenic in animals of various species and both sexes. One cancer study of dogs which evaluated one sex and used one dose level, precluding dose-response evaluation, shows a sufficient number of animals survived to develop tumors.

2.4 LEVELS IN HUMAN TISSUES AND FLUIDS ASSOCIATED WITH HEALTH EFFECTS

No studies were located regarding levels of 3,3'-DCB in human tissues and fluids associated with health effects.

Urinary excretion of 3,3'-DCB and/or metabolites may not persist in man over long periods after initial exposure to the parent compound. Monitoring of urine for 3,3'-DCB and/or metabolites would be useful only if 3,3'-DCB exposure was continuous or, in the case of acute or intermittent exposure, if monitoring was done very early after exposure.

Urinary cystology has been recommended by OSHA as a routine test for 3,3'-DCB exposed workers for the early detection of incipient bladder damage and/or cancer. However, Stula et al. (1978) in his carcinogenicity study of 3,3'-DCB in dogs, reported that results from the annual cystological examination of urine sediment did not provide evidence of genitourinary tract neoplasia even though the bladder cancer

incidence in the dogs surviving until termination of the study was 100%. These findings raise the consideration that urinary cystological examinations alone may not be adequate to detect incipient danger in 3,3'-DCB exposed workers. Since 3,3'-DCB may cause lesions in systems other than the bladder, reliance solely on indices of genitourinary disturbances could be misleading.

2.5 LEVELS IN THE ENVIRONMENT ASSOCIATED WITH LEVELS IN HUMAN TISSUES AND/OR HEALTH EFFECTS

There are no reports directly linking any level of 3,3'-DCB in the environment with a biological effect. However, there appears to be a positive correlation between detectable levels of the chemical in occupational air and its presence in urine, as suggested by the finding in epidemiological studies (Cherniack and Lewis 1984; Handke et al. 1986; London and Boiano 1986; Meigs et al. 1954). Existing data indicate that urinary levels of 3,3'-DCB and/or its metabolites are an indicator of human exposure to 3,3'-DCB. For example, in a survey of a 3,3'-DCB-handling plant, 3,3'-DCB was found in the urine of workers only when their personal breathing-zone air concentrations contained detectable levels of the chemical (London and Boiano 1986). Insufficient information is available to assess the quantitative aspects of the relationship.

2.6 TOXICOKINETICS

Very limited studies exist on the toxicokinetics of 3,3'-DCB in humans. Most data focus on the urinary elimination of the chemical following occupational exposure. Evidence from animal studies suggest that 3,3'-DCB is rapidly absorbed from the gastrointestinal tract. Animals administered a single oral dose of ^{14}C-labelled 3,3'-DCB showed highest concentrations of radioactivity in the liver, kidney, lung, spleen, heart, pancreas and testes. In rats, the major route of elimination of 3,3'-DCB is by metabolism. N-acetyl metabolites (N-acetyl- 3,3'-DCB and N, N'-diacetyl- 3,3'-DCB) have been detected in urine of rats. N-acetyl metabolites are formed *in vivo* under the action of hepatic N-acetyltransferase(s). In man, some isozyme(s) of N-acetyltransferase show marked polymorphic differences; it is thus possible that the proportion of the dose of 3,3'-DCB converted to its N-acetyl metabolites in man may vary widely between individuals. The metabolites undergo rapid excretion primarily into feces and to a lesser extent in urine. Unchanged 3,3'-DCB occurs as a minor urinary excretion product.

2. HEALTH EFFECTS

2.6.1 Absorption

2.6.1.1 Inhalation Exposure

3,3'-DCB has been detected in the urine of workers in 3,3'-DCB-handling plants under conditions which favored inhalation of 3,3'-DCB bound particulate (Meigs et al. 1954; Handke et al. 1986; London and Boiano 1986). However, conditions in the plants were also conducive to dermal exposure. Therefore, the extent to which 3,3'-DCB is absorbed following inhalation exposure in man is inconclusive. It should be noted that there is gastrointestinal exposure as well from breathing dust. The mucociliary clearance mechanism moves most particulate out of the lung and into the gastrointestinal tract. No information was located on absorption in animals following 3,3'-DCB inhalation exposure.

2.6.1.2 Oral Exposure

No data were located on the absorption of 3,3'-DCB following oral exposure in humans. The absorption of orally administered 3,3'-DCB in rats was studied by Hsu and Sikka (1982). Following a dose of 40 mg/kg, 3,3'-DCB attained a peak plasma concentration of 1.25 μg/ml at 8 hr, suggesting that 3,3'-DCB is rapidly absorbed following oral exposure. Further, about 90% of the administered radioactivity was excreted in feces and urine within 72 hours largely as metabolites, indicating a high bioavailability, typical of primary arylamines. The elimination is biphasic, with half-lives of 6 hrs and 14 hrs for the rapid and slow phases (Hsu and Sikka 1982).

2.6.1.3 Dermal Exposure

No studies were located regarding absorption of 3,3'-DCB following dermal exposure in humans. Because of large particle size and increased usage of closed systems and protective clothing, dermal absorption is minimized except under conditions of high humidity and high temperature. In animal studies, Shah and Guthrie (1983) applied ^{14}C-3,3'-DCB in acetone to the shaved skin of rats. Based on the amount of radioactivity remaining at the site of application, dermal absorption at 1, 8, and 24 hr following application was estimated to be 6%, 23% and 49%, respectively.

2.6.2 Distribution

2.6.2.1 Inhalation Exposure

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

2.6.2.2 Oral Exposure

No studies were located regarding the distribution of 3,3'-DCB in humans following oral exposure. Hsu and Sikka (1982) studied the distribution of radioactivity in rat tissues after the oral administration of ^{14}C-3,3'-DCB. Twenty-four hours after a single oral dose, the highest levels of radioactivity were found in the liver, followed by the kidney, lung, spleen, heart, pancreas and testes, in that order. This pattern did not depend on dose. After 96 hours, tissues that retained more than 0.02% of the administered radioactivity were liver (1.48%), muscle (0.37%), kidney (0.19%) and lung (0.02%). Erythrocytes retained more of the radioactivity than lung, but attention was not paid to the hematopoietic system in this study. The effect of repetitive 3,3'-DCB administration on tissue levels of the radioactivity was also studied by Hsu and Sikka (1982). Radioactivity in tissues of animals that received six daily doses of the chemical were generally three to four times as high as the radioactivity in tissues of animals that received a single dose. Similarly, the decline of radioactivity in tissues was generally higher in animals that received a single dose than in those treated with multiple doses of the chemical. The authors concluded that repeated dosing with the chemical did not result in a substantial retention of ^{14}C, and the chemical may be considered to have a fairly low tendency to accumulate in tissues following repetitive dosing.

2.6.2.3 Dermal Exposure

No studies were located regarding distribution of 3,3'-DCB in humans following dermal exposure. The distribution of ^{14}C-3,3'-DCB in rat tissues following dermal application was studied by Shah and Guthrie (1983). Tissues retaining >0.1% of the administered radioactivity 24 hours after application were liver (4.09%), blood (0.75), and lung (0.45). The level in the lung was the same at the 8 and 24 hour time points. Differences in the tissue distribution pattern of total radioactivity between the oral and dermal routes of 3,3'-DCB administration may be presumed to reflect differences in the rates of absorption from these sites. These differences mean that the target

organ in which 3,3'-DCB exerts an adverse effect may depend on the route of exposure to the chemical. Organ toxicity can be better evaluated in comparative studies designed to test tissue distribution and persistence of the chemical using the oral, dermal and inhalation routes of exposure.

2.6.3 Metabolism

2.6.3.1 Inhalation Exposure

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

2.6.3.2 Oral Exposure

No studies were located regarding metabolism of 3,3'-DCB in humans following oral exposure. Studies in animals indicate 3,3'-DCB is extensively metabolized. Hsu and Sikka (1982) reported that bile and urine of rats given a single oral dose of ^{14}C-3,3'-DCB (40 mg/kg/day) contained five metabolites in addition to 3,3'-DCB. None of the metabolites were identified, but a majority were reported to be conjugates. Tanaka (1981) reported that a 24 hr urine sample of rats given a single oral dose of 3,3'-DCB (50 mg/kg/day) contained unchanged 3,3'-DCB, N, N'-diacetyl 3,3'-DCB and N-acetyl 3,3'-DCB in a ratio of 1:3:10.

2.6.3.3 Dermal Exposure

No studies were located regarding metabolism of 3,3'-DCB in humans following dermal exposure. In a 24 hr urine sample of rats given a single dermal application of 3,3'-DCB (50 mg/kg/day), N, N'-diacetyl 3,3'-DCB (but not N-acetyl 3,3'-DCB or the unchanged chemical) was detected (Tanaka 1981). Since the mutagenicity of diacetylated product is much less than either the monoacetylated or parent compound (Lazear et al. 1979; Reid et al. 1984; Tanaka 1981), diacetylation may be a detoxification reaction for 3,3'-DCB.

2.6.4 Excretion

2.6.4.1 Inhalation Exposure

Less than 0.2 ppb 3,3'-DCB was detected in urine samples of 36 workers exposed to 3,3'-DCB-derived pigments (Hatfield et al. 1982). However, the authors did not clearly identify specific pigments. While

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

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

2.6.4.2 Oral Exposure

No studies were located regarding the excretion of 3,3'-DCB in humans following oral exposure. Studies on the fate of 3,3'-DCB-derived pigments fail to provide conclusive evidence that these pigments are broken down to release free 3,3'-DCB in humans. Results from animal studies show that 3,3'-DCB is excreted primarily in feces and to a lesser extent in urine. In rats administered a single oral dose of ^{14}C-3,3'-DCB (40 mg/kg), approximately 58 to 72% of the administered dose was recovered in bile and feces and 23 to 33% in urine (Hsu and Sikka 1982). Most of the material found in bile and feces consisted of conjugated metabolites, while most of the material in urine consisted of nonconjugated metabolites. The elimination appears to be biphasic, with half-lives of about 6 hr and 14 hr for the rapid and slow phases, respectively (Hsu and Sikka 1982). No detectable residues of 3,3'-DCB were found in urine samples of hamsters administered a single dose of 100 mg/kg purified yellow 12 (NCTR 1979; Nony et al. 1980). Similarly, 3,3'-DCB was not detected in urine samples of mice and rats fed 3,3'-DCB derived pigments (12, 16, and 83) in the diet at concentrations of 0.1% (1,000 ppm), 0.3% (3,000 ppm) and 0.9% (9,000 ppm) for 104 weeks (Leuschner 1978).

2.6.4.3 Dermal Exposure

No studies were located regarding the excretion of 3,3'-DCB in humans following dermal exposure. Fecal excretion in rats at 24 hours following 3,3'-DCB exposure was 20% of the administered dose, with urinary excretion of 8% (Shah and Guthrie 1983).

2.7 INTERACTIONS WITH OTHER CHEMICALS

No data were found regarding the interactive effects of 3,3'-DCB with other chemicals that would be relevant to its toxicity. 3,3'-DCB enhances the carcinogenicity of 2-acetylaminofluorene (2-AAF), and butylhydroxybutyl nitrosamine (BBN) (Ito et al. 1983). Combined feeding of BBN (0.001%) 2-AAF (0.005%) and 3,3'-DCB (0.03%) produced urinary bladder papillomas, but no carcinomas. Sequential administration of BBN (0.01%), nitrofurylthiazolylformamide (FANFT, 0.15%), 2-AAF (0.025%) and

3,3'-DCB (0.03%) produced papillomas and carcinomas (Ito et al. 1983; Tatematsu et al. 1977). No tumors were produced in untreated controls and when the chemicals were administered alone. In this situation with a complex mix of four chemicals, no data are available to suggest effects observed were due to 3,3'-DCB. It should be noted that sequential administration of BBN, FANFT and 2-AAF produced papillomas but no carcinomas. 3,3'-DCB may act as a promoter or enzyme inducer; however additional data are needed.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

No information was located that identified any human population as having above-normal susceptibility to 3,3'-DCB toxicities.

2.9 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 3,3'-dichlorobenzidine is available. Where adequate information is not available, ATSDR, in cooperation with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine these health effects (and techniques for developing methods to determine such health effects). The following discussion highlights the availability, or absence, of information on exposure and toxicity of 3,3'-DCB applicable to human health assessment. A statement of the relevance of identified data needs is also included. In a separate effort, ATSDR, in collaboration with NTP and EPA, will prioritize data needs across chemicals that have been profiled.

2.9.1 Existing Information On Health Effects of 3,3'-DCB

As shown in Figure 2-3, essentially no studies of human exposure to 3,3'-DCB were located by specific routes, except for occupational data on direct dermal effects following dermal exposure. Although there are studies of workers in the United States exposed to 3,3'-DCB, these reports are limited by the fact that exposure potentially involved other chemicals, and both the route and extent of exposure are largely unknown. Dermal effects have also been investigated in experimental animals as well as ocular irritant properties of 3,3'-DCB exposure.

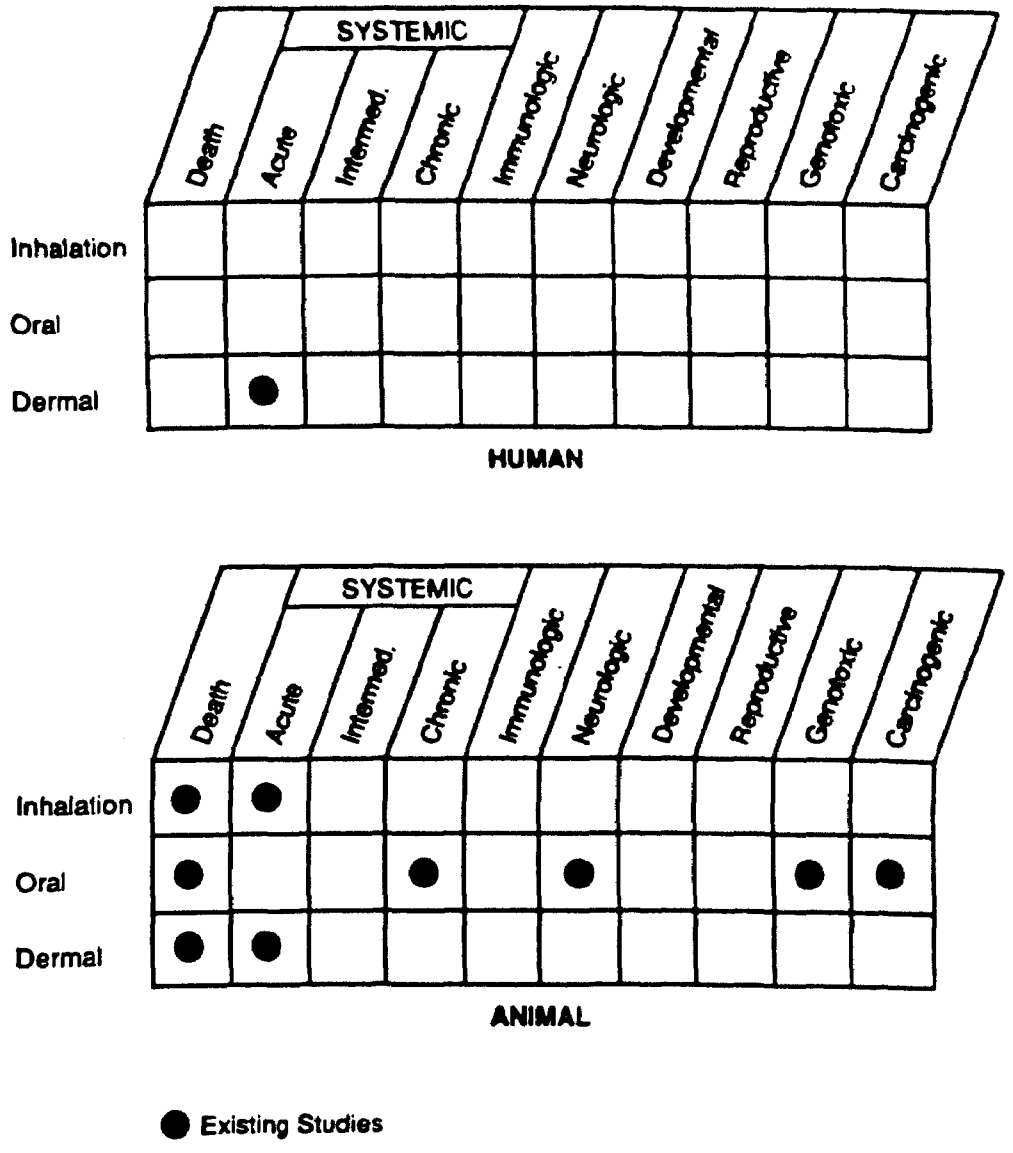


FIGURE 2-3. Existing Information on Health Effects of 3,3'-Dichlorobenzidine

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

2.9.2 Data Needs

Single Dose Exposure. Potential exposure to 3,3'-DCB may occur through inhalation of contaminated airborne dust, ingestion of contaminated water or skin contact. Studies regarding single dose exposure showed the compound can cause eye damage following conjunctival application and possibly respiratory effects when inhaled. The significance of these findings is minor as conjunctival application is not a typical route of exposure for the general population. 3,3'-DCB can be lethal following oral and dermal exposure at high doses. Comprehensive gross and histopathological evaluations have not been conducted and clinical signs have not been monitored. Such studies may provide insight into systemic toxicity and potential health threat associated with one-time exposure.

Repeated Dose Exposure. Repeated dose inhalation studies have been performed in rats without systemic effects, but these studies used only one dose level and the number of animals tested was not specified. No repeat oral or dermal dose studies were found. Animal studies evaluating toxicological parameters at several dose levels would provide dose response data which could prove more predictive when assessing potential adverse effects in humans following repeated exposure.

Chronic Exposure and Carcinogenicity. Long-term exposure of humans to 3,3'-DCB by inhalation and dermal contact may occur in occupational settings and potential for oral exposure exists in areas near hazardous waste sites. Available chronic oral studies provide information regarding systemic and carcinogenic effects in rats and dogs. These studies employed one dose level and toxicological parameters measured were limited. No chronic animal inhalation or dermal exposure studies were located. Well-conducted chronic inhalation, dermal and oral studies involving low-dose exposure in animals might provide dose-response data on potential systemic effects of exposure in humans. Available data do not establish the relationship between the concentration of 3,3'-DCB and/or its metabolites in the body and the probability of cancer. Studies designed to establish urinary excretion levels that are associated with disease may prove useful, but due to the long latency of the possible carcinogenic effects, such studies are difficult to perform.

Genotoxicity. Available studies show that 3,3'-DCB does alter genetic material. Studies involving more predictive indicator test systems may allow a better assessment of genotoxic potential.

Reproductive Toxicity. No studies were found regarding reproductive toxicity of 3,3'-DCB. Well-conducted multigenerational reproductive studies in animals may provide some insight as to the potential effects of 3,3'-DCB on human reproductive processes.

Developmental Toxicity. No studies were found regarding developmental toxicity of 3,3'-DCB in humans. Animal studies have shown that 3,3'-DCB crosses the placenta and can affect the growth of the kidneys after parenteral exposure. The effects of the chemical on development have not been studied following oral, inhalation or dermal exposure. Well-conducted animal studies employing various dose levels and relevant exposure routes during critical developmental periods may provide information on potential fetotoxicity, embryotoxic and teratogenic effects in humans. Further animal data may provide dose-response information if studies are conducted to determine what dose of 3,3'-DCB, or its metabolites, reaches the fetus.

Immunotoxicity. No studies were located determining the role of the immune system during 3,3'-DCB exposure. Investigations in animals on the effects of 3,3'-DCB on the immune system may be valuable since the immune system has been reported to be sensitive to chemical toxicants.

Neurotoxicity. A chronic oral study in dogs examined organs and tissues of the nervous system and reported signs of neuronal degeneration. The confidence in the effect reported is reduced since only one dose level was tested and the effect failed to appear in more than one of the 6 test dogs. Additional studies may verify this finding and document dose-response relationships between exposure by relevant routes to low-dose concentrations and the potential neurotoxicity of 3,3'-DCB.

Epidemiological and Human Dosimetry Studies. The only known health effect associated with DCB exposure in humans is dermatitis which was attributed to a manufacturing process change resulting in exposure of workers to DCB-free base. Epidemiological studies of people who live in areas where 3,3'-DCB has been detected in groundwater, near industries releasing 3,3'-DCB, or near hazardous waste sites, and of occupational

exposure, could provide information on whether 3,3'-DCB exposure produces effects in humans.

No studies were located that monitored human tissues for 3,3'-DCB or its metabolites. 3,3'-DCB is excreted in urine. If 3,3'-DCB and metabolites can be detected and correlated with exposure, it may be possible to monitor humans for exposure. With monitoring, it may be possible to correlate urinary levels of 3,3'-DCB or its metabolites, with systemic effects.

Biomarkers of Disease. There are no disease states in humans that can clearly be associated with exposure to 3,3'-DCB. If comprehensive epidemiological studies are conducted, it may be possible to identify subtle changes, such as altered blood chemistry indices, associated with a particular disease state.

Disease Registries. The only reported health effects from human 3,3'-DCB exposure are skin, eye, nose and throat irritation. If epidemiological studies identify particular diseases attributable to 3,3'-DCB exposure, it may be possible to determine the number of people affected.

Bioavailability from Environmental Media. 3,3'-DCB is unstable in air and water but persists in soil. No studies were located regarding the bioavailability of 3,3'-DCB from these media. The lack of data do not necessarily indicate a lack of bioavailability. 3,3'-DCB reportedly bioconcentrates in the aquatic environment. Analysis of the body fluids of those people who consume fish may allow a determination of exposure, and estimation of the degree of exposure.

Food Chain Bioaccumulation. No studies were located regarding the food chain bioaccumulation of 3,3'-DCB. Based on an assumed log K_{ow} in the range of 3.02 to 3.78, 3,3'-DCB is not likely to bioaccumulate strongly.

Absorption, Distribution, Metabolism, and Excretion. Available data are insufficient to allow accurate evaluation of absorption, metabolism or persistence of 3,3'-DCB in human tissues. Additional studies to identify and quantify metabolites of 3,3'-DCB in humans and animals would be useful in establishing the relevance of animal studies in predicting human health effects. Metabolic handling of 3,3'-DCB in humans may need to be better characterized before urinary levels of the chemical or its metabolites can be used to quantitate human exposure.

Comparative Toxicokinetics. Pharmacokinetics studies have not been performed under conditions analogous to those of the carcinogenicity studies. Therefore, it is not possible to determine systemic levels of the chemical associated with the reported effects. Pharmacokinetics data concomitant with an identifiable biological effect would markedly increase accuracy and improve species extrapolation when evaluating the true potency of 3,3'-DCB in respect to that specific effect.

2.9.3 Ongoing Studies

Dr. M. Iba (Rutgers University) is performing research directed at the identification of the *in vivo* and *in vitro* metabolites of DCB. No other ongoing studies were located.

3. CHEMICAL AND PHYSICAL INFORMATION

3.1 CHEMICAL IDENTITY

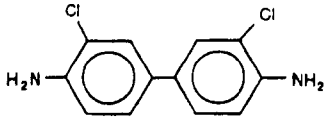
Table 3-1 lists common synonyms, trade names and other pertinent identification information for 3,3'-DCB.

3.2 PHYSICAL AND CHEMICAL PROPERTIES

Table 3-2 lists important physical and chemical properties of 3,3'-DCB.

3. CHEMICAL AND PHYSICAL INFORMATION

TABLE 3-1. Chemical Identity of 3,3'-Dichlorobenzidine

	Value	References
Chemical Name	3,3'-Dichlorobenzidine	NLM 1988
Synonyms	Dichlorobenzidine; 3,3'-Dichloro-4,4'-Diaminobiphenyl; 4,4'-Diamino-3,3'-Dichlorobiphenyl; o,o'-Dichlorobenzidine	
Trade Name	curithane	IARC 1982a
Chemical Formula	$C_{12}H_{10}Cl_2N_2$	
Chemical Structure		EPA 1983
Identification Numbers:		
CAS Registry	91-94-1	NLM 1988
NIOSH RTECS	DD0525000	HSDB 1988
EPA Hazardous Waste	U073	NLM 1988
OHM-TADS	8100004	HSDB 1988
DOT/UN/NA/IMCO Shipping	ND	HSDB 1988
HSDB	1632	NLM 1988
NCI	ND	

NLM - National Library of Medicine; IARC - International Agency for Research on Cancer; EPA - Environmental Protection Agency; CAS - Chemical Abstracts Service; NIOSH - National Institute for Occupational Safety and Health; RTECS - Registry of Toxic Effects of Chemical Substances; HSDB - Hazardous Substances Data Bank; OHM-TADS - Oil and Hazardous Materials/Technical Assistance Data System; DOT/UN/NA/IMCO - Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; ND - No data; NCI - National Cancer Institute.

3. CHEMICAL AND PHYSICAL INFORMATION

TABLE 3-2. Physical and Chemical Properties of 3,3'-Dichlorobenzidine

Property	Value	References
Molecular Weight	253.13	Verschueren 1977
Color	grey to purple crystalline	HSDB 1988
Physical State	solid	HSDB 1988
Melting Point, °C	132 (up to 136)	Mabey et al. 1982; DCMA 1989
Boiling Point, °C	368 (estimate)	PCGEMS 1988
Density	ND	HSDB 1988
Odor	mild	HSDB 1988
Odor Threshold Water Air, ppm	ND	HSDB 1988
Solubility in dist Water, mg/L	3.11	DCMA 1989
Organic Solvents	soluble in alcohol, benzene	HSDB 1988
Partition coefficients Log octanol/water	3.5	Mabey et al. 1982; DCMA 1989
Log k(organic carbon/water)	3.2	Mabey et al. 1982
Vapor Pressure, mm Hg	4.5E-09 at 20°C	DCMA 1989
Henry's law constant, $atm\text{-}m^3/mol$	5E-10	DCMA 1989
Ignition temperature, °C	350	DCMA 1989
Flash point	≥200	DCMA 1989
Flammability limits	ND	HSDB 1988
Conversion factors ppm (v/v) to mg/m^3 in air (20°C)	1 ppm = 10.4 mg/m^3	
mg/m^3 to ppm (v/v) in air (20°C)	1 mg/m^3 = 0.10 ppm	

HSDB = Hazardous Substances Data Bank; PCGEMS = Personal Computer Conversion of Graphical Exposure Modeling System; DCMA = Dry Color Manufacturers' Association; ND = no data; dist = distilled.

4. PRODUCTION, IMPORT, USE AND DISPOSAL

4.1 PRODUCTION

3,3'-DCB is commercially produced through various reduction procedures of o-nitrochlorobenzene to form a hydrazo compound which is rearranged in the presence of mineral acids to form 3,3'-DCB (DCMA 1989; Sax 1987). Commercial supplies are usually provided in the form of the dihydrochloride salt because of its greater stability.

A small number of domestic companies are listed as manufacturers. Current production volumes of 3,3'-DCB are considered confidential business information and cannot be reported. The United States International Trade Commission (USITC 1984a) reported a 1983 production volume of 3,3'-DCB-based dyes of over 18 million pounds. Consumption of 3,3'-DCB in the United States amounted to 9,900,000 pounds in 1987 (Hopmeier 1988).

4.2 IMPORT

Imports of 3,3'-DCB base and salts were 1.1 million pounds in 1983, while pigments were about 129,000 pounds in 1983 (USITC 1984b).

4.3 USE

3,3'-DCB is used primarily in the production of yellow, and some red and orange pigments for the printing ink, textile, paper, paint, rubber, plastic and related industries (EPA 1979). As of 1983, 7 specific pigments were commercially available. Little, if any, dye is prepared from this compound. The chemical also has application as a compounding ingredient for rubber and plastics, and can be used to test for the presence of gold (Searle Chemical Carcinogens 1976). 3,3'-DCB is used in the formulation of the raw material tetraminobiphenyl which is used to produce polybenzimidazole (PBI). PBI fiber is used in many protective clothing applications, such as fireman's apparel, welder's garments, high temperature gloves and crash rescue garments (Celanese 1985).

4.4 DISPOSAL

3,3'-DCB is treated in the workplace as a controlled substance under OSHA. Therefore, strict requirements have been made to minimize exposure to the chemical in the workplace air and contact with the skin and eye. Nonetheless, some releases may occur in wastewater effluents.

A report by London and Boiano (1986) indicates that one company which purchases 3,3'-dichlorobenzidine as the dihydrochloride salt in sealed fiber in drums rinses the empty drums with water, adds the rinse water to the product stream, then sprays the drums with a sodium hypochlorite bleach solution (converting 3,3'-DCB to a quinone-type compound), and places them in polyethylene bags for disposal.

4.5 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 3,3'-dichlorobenzidine is available. Where adequate information is not available, ATSDR, in cooperation with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine these health effects (and techniques for developing methods to determine such health effects). The following discussion highlights the availability, or absence, of exposure and toxicity information applicable to human health assessment. A statement of the relevance of identified data needs is also included. In a separate effort, ATSDR, in collaboration with NTP and EPA, will prioritize data needs across chemicals that have been profiled.

4.5.1 Data Needs

According to the Emergency Planning and Community Right to Know Act of 1986 (EPCRTKA), (§313), (Pub. L. 99-499, Title III, §313), industries are required to submit release information to the EPA. The Toxic Release Inventory (TRI), which contains release information for 1987, became available in May of 1989. This database will be updated yearly and should provide a more reliable estimate of industrial production and emission.

Production, Use, Release and Disposal. Recent USITC data do not give current production volumes specifically for 3,3'-DCB. If one can infer from production data for specific pigments, production is only slightly elevated from a decade ago. Import figures from the late 1970s show wide fluctuation. It is possible that the basis of the figures used varies.

Use data appear adequate, as 3,3'-DCB is largely a single-purpose compound. Regulated disposal practices were not specified, although an example of current disposal practice was obtained.

5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Major release routes of 3,3'-DCB to the environment appear to be wastewaters, sludges, and solid wastes where emissions are not properly controlled during the production and use of 3,3'-DCB and benzidine-based dyes. The chemical was detected in ground and surface water at less than one percent of a sample of hazardous waste sites but in the soil of 4.4% (CLPSD 1988). Relatively high concentrations have been found in the vicinity of industrial sources such as those resulting from the improper land disposal of 3,3'-DCB solid wastes.

Concern for human health is primarily for inhalation of airborne dust, drinking of contaminated well water by persons living in the proximity of hazardous waste sites, and skin contact by workers in occupational settings. However, occupational case reports suggest that risk to workers exposed to 3,3'-DCB through the use of benzidine-based dyes may be minimal. No adverse health effects were reported in an average of 20 workers engaged in the manufacture and handling of 3,3'-DCB alone (concentration not specified) in a Japanese facility (DCMA 1989). Less than 0.2 ppb 3,3'-DCB was detected in urine samples of 36 workers exposed to 3,3'-DCB-derived pigments (specific pigments not specified) (Hatfield et al. 1982). While these data suggest that 3,3'-DCB derived pigments are not metabolized in humans, limitations in the existing evidence do not allow a conclusive decision about the human health implications.

3,3'-DCB readily photolyzes in water exposed to light, but may not readily biodegrade in soil and acclimated sludges. It has a strong tendency to partition to soils and sediments which reduces the potential for human exposure (Boyd et al. 1984; Chung and Boyd 1987; Sikka et al. 1978). It does not volatilize or hydrolyze in solution, but it may slowly oxidize (Banerjee et al. 1978; Callahan et al. 1979). 3,3'-DCB may be bioconcentrated by aquatic organisms (Appleton and Sikka 1980), but it is not certain if it is biomagnified by transfer through the food chain.

5.2 RELEASES TO THE ENVIRONMENT

5.2.1 Air

3,3'-DCB is handled by industry primarily as a powder or a paste (NIOSH 1980). 3,3'-DCB is not a volatile chemical. A vapor pressure of 4.5×10^{-9} mmHg has been reported (DCMA 1989). Prior to OSHA 1974 regulations, benzidine and 3,3'-DCB were manufactured in open systems

that permitted atmospheric releases of suspended particles at the work site (Shriner et al. 1978), but no data were located specifically for 3,3'-DCB emissions (atmospheric or in water). The absence of data may be attributed to then-used analytical methods that could not distinguish benzidine from its derivatives or many other aromatic amines (Shriner et al. 1978). Under OSHA regulations adopted in 1974, only closed systems are permitted, and atmospheric emissions are presumably reduced because of this regulation. Aggregate annual releases of DCB to the air in the United States are estimated to be far less than 22 pounds, and possibly lower than one pound (DCMA 1989).

5.2.2 Water

The base form of 3,3'-DCB is sparingly soluble in water. Banerjee et al. (1978) measured the solubility of 3,3'-DCB•2HCl in water as 4 mg/L (pH 6.9). 3,3'-DCB may be released into the environment in wastewaters generated by the production of dyes and pigments. Preliminary data from the Contract Laboratory Program Statistical Data Base (CLPSD 1988) indicated that 3,3'-DCB was detected in ground and surface water samples collected at hazardous waste sites. The frequency of detection was only 0.6% and 0.3%, respectively, at over 500 sites. Median concentrations were not available.

5.2.3 Soil

Soils and other unconsolidated materials may be contaminated with 3,3'-DCB by atmospheric transport of dust particles, industrial discharges, or by 3,3'-DCB-contaminated wastewater sludge. However, there is a paucity of data to show how frequently contamination occurs. Boyd et al. (1984) reported that the improper disposal of industrial waste sludges containing 3,3'-DCB resulted in soil, groundwater and surface water contamination. The sludges had been placed into earthen pits. Soil, groundwater and surface water contamination at other hazardous waste sites has also been detected. Preliminary data from the Contract Laboratory Program Statistical Data Base (CLPSD 1988) indicated that 3,3'-DCB was detected in solid phases collected at hazardous waste sites. The frequency of detection was 4.4% at over 500 sites.

5.3 ENVIRONMENTAL FATE

Because 3,3'-DCB stays attached to airborne dust particles or is bound to particulate matter, it is subject to dispersion, gravitational settling, and wash out by rain. In water, 3,3'-DCB is sparingly soluble, does not volatilize or hydrolyze, and may slowly oxidize in

solution (Banerjee et al. 1978; Callahan et al. 1979; Mabey et al. 1982). 3,3'-DCB may be strongly adsorbed by soils, clays, and sediments, depending on the pH of the soil-water system. It may be strongly bound by soil organic matter (Boyd et al. 1984; Chung and Boyd 1987; Sikka et al. 1978). It does not appear to be readily biodegradable in soil or wastewater sludges. 3,3'-DCB may be bioconcentrated by aquatic organisms under experimental conditions (Appleton and Sikka 1980), but it is not certain if it is bioaccumulated or transferred through the food chain.

5.3.1 Transport and Partitioning

In the atmosphere, 3,3'-DCB stays attached to dust particles or bound to particulate matter. As such, suspended 3,3'-DCB is subject to atmospheric convection, dispersion, gravitational settling, and wash-out by rain.

The Henry's Law constant (K_H) for a compound is useful in estimating the partitioning of the compound between its vapor phase and aqueous media. A value of 5×10^{-10} atm-m^3/mole has been estimated (DCMA 1989). This very low value suggests that 3,3'-DCB essentially remains dissolved in water, and does not migrate from water into air.

3,3'-DCB in solution has a strong tendency to be adsorbed onto soils and sediments. The extent of adsorption of hydrophobic (sparingly-water soluble) compounds has been shown to be highly correlated with the organic carbon content of the adsorbents (Hassett et al. 1983). When adsorption is expressed as a function of organic carbon content, an organic carbon-water partition coefficient (K_{oc}) is generated which is a unique property of the compound and may be used to rank the relative mobility of organic contaminants in saturated soil-water systems. Mabey et al. (1982) calculated a K_{oc} value for 3,3'-DCB of 1553, based on an octanol-water partition coefficient (K_{ow}) of 3236. This relatively high value implies that 3,3'-DCB would exhibit "low mobility" in soil (see Roy and Griffin 1985). However, 3,3'-DCB is not strictly a hydrophobic compound but can exist as a weak base in water, and exists in both neutral and cationic forms. Written as a hydrolysis reaction, the amine groups may be protonated as follows:

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

The PKa's of the conjugate acids ($DCBH^+$ and $DCBH_2^{2+}$) are apparently not known accurately; Sikka et al. (1978) and Boyd et al. (1984)

reported that they are less than 4. In the pH range of most environmental situations (pH 6 to 8), the dominant state of 3,3'-DCB in water is as the non-ionic form. As pH increases, the proportion of cationic 3,3'-DCB decreases, and the extent of adsorption to sediments via Coulombic interactions would also decrease and 3,3'-DCB adsorption would be dominated by hydrophobic processes. This expectation was demonstrated by Sikka et al. (1978), who found that the adsorption constant (K_f) decreased with increasing pH, especially in the range of pH 7 to 9. The adsorption data conformed to the Freundlich equation, viz. $C_a = K_f C_s^{1/n}$ where Ca is the concentration of DCB adsorbed per mass of adsorbent, and C_s is the equilibrium concentration of 3,3'-DCB in solution K_{fd} and 1/n are empirically-derived constants. Sikka et al. (1978) and Boyd et al. (1984) found no correlation between K_f and the organic carbon content of the sediments. In the same regard, the extent of benzidine adsorption does not correlate to the organic carbon content of soils and sediments (Graveel et al. 1986; Zierath et al. 1980). Boyd et al. (1984) concluded that nonionized 3,3'-DCB is subject to hydrophobic bonding to some extent. It is clear from these studies that adsorption constants for 3,3'-DCB cannot be accurately predicted for a given soil based only on a K_{oc} value.

The adsorption of 3,3'-DCB by soils and sediments may not be reversible (Boyd et al. 1984; Chung and Boyd 1987; Sikka et al. 1978). The extent of 3,3'-DCB desorption decreased with an increase in the age of the sample. Also, the adsorbed 3,3'-DCB was resistant to extraction; after 24 hours of 3,3'-DCB-sediment contact, only 36% of the parent compound could be extracted by methanol. Both Sikka et al. (1978) and Boyd et al. (1984) speculated that 3,3'-DCB forms covalent bonds with soil humic components. Experiments have indicated that covalent binding of ring-substituted anilines to humates is not a readily reversible reaction (Parris 1980). 3,3'-DCB was highly immobile in soil column experiments (Chung and Boyd 1987). Water was passed through sandy soil (Entic Haplorthod), and a 3,3'-DCB-contaminated sewage sludge samples. Most of the 3,3'-DCB was bound by the soil, while sludge and leachate samples collected from the columns contained low concentrations of 3,3'-DCB. This information suggests that DCB would migrate from contaminated sludge to soil.

Since 3,3'-DCB is lipophilic, it may be concentrated from solution by aquatic organisms. Bluegill sunfish were exposed to radiolabeled 3,3'-DCB in dynamic flow experiments for 120 to 168 hours by Appleton and Sikka (1980). Moderately low bioconcentration factors (BCF) of 495 to 507 were calculated for the whole fish. Freitag et al. (1984, 1985) reported a bioconcentration factor in fish (golden ide) of 610 and in

green algae of 940. EPA (1980b) reported a bioconcentration factor in edible portions of bluegill sunfish of 114-170. Bioaccumulation by plants or animals has not been studied. Assuming a Log K_{ow} in the range 3.02 to 3.78 (DCMA 1989; Mabey et al. 1982), 3,3'-DCB is not likely to bioaccumulate strongly.

5.3.2 Transformation and Degradation

5.3.2.1 Air

3,3'-DCB in the atmosphere may be photooxidized with hydroxyl radicals and ozone, but there were no quantitative data on reaction rates. Radding et al. (1977) estimated the persistence of "all benzidines" in the atmosphere by assuming a hydroxyl radical concentration of 8×10^{-15} mole/liter (an average value in a 24-hour day-night cycle). Treating the photooxidation process as a first-order reaction, the rate constant was 7.2×10^{12}/mole-hr and the corresponding half-life was 12 hours. This approach was based on data on the rates of reaction of hydroxyl radicals with olefins, aromatics, and alkanes in the atmosphere. The estimated half-life of 3,3'-DCB in air has ranged from 1 to 60 days (Shriner et al. 1978; EPA 1980b). Based on the reaction rate constant of photodegradation in the atmosphere, the half-life may be as little as two hours (DCMA 1989). There was no other information on the fate of atmospheric 3,3'-DCB.

5.3.2.2 Water

The limited information that is available suggest that 3,3'-DCB may photolyze yielding benzidine which is more photostable. It does not appear that the chemical is susceptible to other transformations in water.

There are no data to suggest that the hydrolysis of 3,3'-DCB is significant (Callahan et al. 1979). Mabey et al. (1982) proposed a hydrolysis-rate constant of 0/mole-hour for 3,3'-DCB.

It has been speculated that aromatic amines can be oxidized in solution by organic radicals, but there are no actual data on reaction rates. Based on structural analogs, Radding et al. (1977) estimated that the half-life of such compounds in water is approximately 100 days, assuming a peroxy concentration of 10^{-10} mole/L in sunlit, oxygenated water. Based on the oxidation rates with similar compounds, Mabey et al. (1982) treated the direct oxidation of 3,3'-DCB by oxygen in solution as a first-order reaction, and estimated a reaction rate

constant of less than 4 x 10^7/mole-hour. The oxidation rate constant with peroxy radicals was estimated to be approximately 4 x 10^7/mole-hour. However, no information was located that demonstrates that 3,3'-DCB is significantly oxidized in water.

In a study reported by Sikka et al. (1978) and Banerjee et al. (1978), 3,3'-DCB was found to be extremely photolabile in water. 3,3'-DCB photolyzed yielding monochlorobenzidine, benzidine, and a number of colored, water-insoluble products. In natural sunlight, the half-life of 3,3'-DCB in water was approximately 90 seconds. While 3,3'-DCB is very rapidly photolyzed under environmental conditions, the process may yield benzidine, a relatively photostable carcinogen (Banerjee et al. 1978).

3,3'-DCB was not metabolized by microorganisms over a four-week period in lake water samples (Sikka et al. 1978). The sample from one of two reservoirs studied contained approximately 5 x 10^6 cells/mL, but the composition of the biological community was not described. Minor decreases in 3,3'-DCB concentrations were attributed to adsorption onto suspended sediment.

5.3.2.3 Soil

It does not appear that 3,3'-DCB is significantly degraded in soil nor that it is transformed to other forms.

Unsubstituted benzidine may be oxidized at clay surfaces when mixed with some types of clay minerals (Tennakoon et al. 1974; Theng 1971). Benzidine is oxidized to a monovalent radical cation by iron (III) in the silicate lattice and by aluminum at crystal edges. However, there is no experimental evidence that demonstrates that 3,3'-DCB is subject to the same type of surface oxidation at solid-liquid interfaces.

Activated sludge did not degrade 3,3'-DCB after weekly subculturing. The sludge was not described or chemically characterized. Observed decreases in 3,3'-DCB concentration were attributed to adsorption by the sludge.

Brown and Laboureur (1983) summarized the results of seven laboratories conducting aerobic biodegradation experiments with 3,3'-DCB. There was a clear dependence of the extent of degradation on the concentration of yeast extract added to the batch containers. The role of the extract was uncertain, but without it, no degradation was detected. Brown and Laboureur (1983) felt that these results showed the

"inherent biodegradability" of 3,3'-DCB, but that it would not be classified as "readily biodegradable." Possible degradation mechanisms, and degradation by-products were not discussed.

3,3'-DCB degraded very little when incubated with soil. In a study by Boyd et al. (1984), a Brookston clay loam soil (a typic Argiaquoll) was incubated aerobically and anaerobically in batch experiments. Under aerobic conditions, 3,3'-DCB degradation occurred at a very slow rate; accumulative $^{14}CO_2$ production was approximately 2% after 32 weeks. Under anaerobic conditions, no gas evolution was detected after one year of incubation. Boyd et al. (1984) did not comment on the population or type of microorganisms in the soil sample. Additional studies by Chung and Boyd (1987) indicated that 3,3'-DCB was very persistent in soil and sludge-amended soil. Biodegradation of ^{14}C-labeled 3,3'-DCB was evaluated during a 182-day incubation period in a sandy soil (Entic Haplorthod) amended with sewage sludge. The total amount of ^{14}C-3,3'-DCB recovered as $^{14}CO_2$ was less than 2%. It should be noted that biodegradation when measured by $^{14}CO_2$ evolution may be a conservative estimate of the extent of decomposition. This technique does not account for carbon that is incorporated into the biomass or into soil organic matter, or if the compound is only partially metabolized (Graveel et al. 1986).

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

3,3'-DCB was not detected in ambient air at production facilities at detection limits of 0.1-5.0 ng/m^3 (Narang et al. 1982; Riggin et al. 1983). The median concentration of 3,3'-DCB in waste effluents (< 10 ppb), groundwater (< 10 ppb), surface water (< 10 ppb), and soils (< 1 ppb) is very low, although significant contamination may be associated with hazardous waste sites (Staples et al. 1985). Moreover, the production and utilization of benzidine-based dyes has decreased in the last 30 years, while environmental and health regulations have been implemented to reduce the release of 3,3'-DCB to the environment. However, the inability to determine very low concentrations in water creates some uncertainty in estimating levels in the environment.

5.4.1 Air

3,3'-DCB does not naturally occur in the environment (IARC 1982a). 3,3'-DCB was not detected in ambient air of two dyestuff production plants at detection limits of 5 (Narang et al. 1982) and 0.1 ng/m^3 (Riggin et al. 1983). Current data on occupational exposure levels

indicate levels ≤ 0.6 - 2.5 $\mu g/m^3$ in 3,3'-DCB production and pigment manufacturing plants in Germany (DCMA 1989).

5.4.2 Water

Staples et al. (1985) used EPA's computerized water quality data base (STORET) to determine the median concentration of 3,3'-DCB in surface water, groundwater, and in municipal and industrial inflow and outflow. The median concentration of 3,3'-DCB in 1,239 samples of waste effluent, collected from about 1980 to 1984, was reported to be less than 10 ppb. 3,3'-DCB was detected in only 12 samples. The median concentration of 3,3'-DCB in both surface and groundwater was also reported to be less than 10 ppb. The EPA (1980b) reported that water samples collected from drinking water wells near a waste disposal lagoon that contained 3,3'-DCB-manufacturing wastes had concentrations of the chemical ranging from 0.13 to 0.27 ppm. EPA (1983) indicated that 3,3'-DCB concentrations in wastewaters from metal finishing operations were 0.07 ppb or less. Discharge concentrations from other industrial sources were at most 10 ppb.

5.4.3 Soil

Staples et al. (1985) reported that the median concentration of 3,3'-DCB in sediments in the United States was estimated to be less than 1 ppm dry sediment. In 347 measurements recorded in the STORET data base, none of the samples contained detectable concentrations of 3,3'-DCB.

5.4.4 Other Media

There is a potential for 3,3'-DCB to occur in wastewater sludges and industrial solid wastes. A 3,3'-DCB concentration in sludge of 16 ppm has been reported (Chung and Boyd 1987). 3,3'-DCB (3.13 mg/kg of sewage sludge, dry weight) was detected in two cases out of a total of 253 sewage treatment plants (Fricke et al. 1985). Concentrations up to 535 $\mu g/L$ were detected in communal sewage treatment plant (Lopez-Avila et al. 1981). The chemical was detected at 8.55 mg/kg in sewage sludge of an aeration basin (Demirjian et al. 1984). It is very unlikely that 3,3'-DCB occurs in food in general, since the chemical has no agricultural or food chemical application. However, the chemical has been detected in fish in an experimental exposure study. 3,3'-DCB (^{14}C) was found to rapidly accumulate in bluegill sunfish as a result of exposure to water containing 5 ppb or 0.1 ppm of the chemical. Residues were distributed in both the edible and nonedible portions (Appleton and

Sikka 1980). However, 3,3'-DCB was not detected in fish samples obtained from rivers near nine textile and dyestuff manufacturers known to use 3,3'-DCB (Diachenko 1979).

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

In most cases, benzidine and its congeners such as 3,3'-DCB are potential hazards only in the vicinity of dye and pigment plants where wastes may escape or be discharged (EPA 1980b; Shriner et al. 1978). The risk to the general population from 3,3'-DCB is unknown, but based on available data, the potential for nonindustrial exposure via air, soil, or water is expected to be negligible. However, the greatest risk of exposure to the general public is from the improper land disposal of industrial wastes generated by the synthesis and use of 3,3'-DCB compounds. The significance of this risk can only be evaluated on a site by site basis.

A potential source of exposure to 3,3'-DCB by the general public may be by the use of pressurized spray containers of paints, lacquers, and enamels containing traces of benzidine yellow, an azo dye derived from 3,3'-DCB (Shriner et al. 1978). No quantitative data were located regarding use of 3,3'-DCB in consumer products.

The most likely occupational health risks exist in the processing of 3,3'-DCB in the synthesis of azo dyes, and for workers in the garment, leather, printing, paper, and homecraft industries where benzidine-based dyes are used. However, there appears to be no information available on current levels of occupational exposure in the United States. While there is no evidence for <u>in vivo</u> cleavage of 3,3'-DCB-derived pigments to free DCB in animals, it cannot be concluded that 3,3'-DCB-derived pigments are not metabolized in humans.

5.6 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

NIOSH (1980) concluded that during the use of benzidine-based dyes, the greatest potential for exposure would be expected to be by dermal absorption or inhalation by personnel who routinely handle dry powders. However, EPA (1980b) has generalized that dermal absorption in the workplace was probably a minor route of 3,3'-DCB exposure, although dermatitis has occurred in plants where 3,3'-DCB and 3,3'-DCB-based pigments were manufactured. It may be that health risks with regard to 3,3'-DCB exposure depend on the specific operations of the individual plant, and extent of personal protective practices of the individual operator.

5.7 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 3,3'-dichlorobenzidine is available. Where adequate information is not available, ATSDR, in cooperation with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine these health effects (and techniques for developing methods to determine such health effects). The following discussion highlights the availability, or absence, of exposure and toxicity information applicable to human health assessment. A statement of the relevance of identified data needs is also included. In a separate effort, ATSDR, in collaboration with NTP and EPA, will prioritize data needs across chemicals that have been profiled.

5.7.1 Data Needs

Physical and Chemical Properties. It has been demonstrated that 3,3'-DCB is strongly adsorbed by soils and sediments, and that it may not readily desorb. Adsorption can not be accurately predicted a priori; such data are soil-system specific and must be determined experimentally for each system under study.

Environmental Fate. It is not known if 3,3'-DCB, like benzidine, is oxidized by clay minerals or if cations in water can have the same effect. 3,3'-DCB does not appear to easily biodegrade, but the few studies in this area did not state the type(s) or concentrations of microorganisms used in each study. More systematic studies with other organisms may prove useful.

Exposure Levels in Environmental Media. There were no data on current levels of atmospheric emissions of 3,3'-DCB or its potential to act as a surface contaminant of soil environments. It is difficult to determine 3,3'-DCB levels in the aquatic environment because the concentrations tend to be at or below analytical detection limits. In general, it may only be possible to fully ascertain the environmental fate of 3,3'-DCB with analytical advances that permit the routine determination of very low concentrations. Moreover, it would help to determine the nature and environmental fate of breakdown products of 3,3'-DCB.

Exposure Levels in Humans. It has been speculated that the 1974 OSHA regulations have reduced workplace air levels of 3,3'-DCB. It would be helpful to conduct exposure studies to monitor air levels in the workplace. It would be beneficial to assess how chemical spills are handled. There is a lack of information on the extent of air, water, and soil contamination by industrial wastes containing 3,3'-DCB.

Exposure Registries. An exposure registry for 3,3'-DCB was not located.

5.7.2 Ongoing Studies

No information was located regarding on-going studies.

6. ANALYTICAL METHODS

6.1 BIOLOGICAL MATERIALS

The major ways of determining 3,3'-DCB and its congeners have been summarized by Fishbein (1984), and means for the derivatization of 3,3'-DCB and its metabolites in biological materials and determination by GC and HPLC have been described by Bowman and Nony (1981), Nony and Bowman (1980) and Nony et al. (1980). Most studies of the analysis of 3,3'-DCB in biological materials have concentrated upon urine as a means of monitoring human exposure. In addition to 3,3'-DCB itself, its metabolites have been determined in biological samples. These include monoacetylated metabolites and diacetylated metabolites (Bowman and Nony 1981). The analytical methods used to measure 3,3'-DCB and its metabolites in biological materials include gas chromatography (NIOSH 1985) and liquid chromatography with ultraviolet absorption or electrochemical detection (Trippel-Schulte et al. 1986). 3,3'-DCB can be extracted from urine with a solvent such as benzene and may be converted to volatile derivatives such as heptachlorobutyl dichlorobenzidine (Bowman and Nony 1981) for gas chromatographic determination.

Methods for the determination of 3,3'-DCB in biological materials are summarized in Table 6-1.

6.2 ENVIRONMENTAL SAMPLES

Analysis of 3,3'-DCB in environmental samples is most commonly achieved by gas chromatography/mass spectrometry (GC/MS) (EPA 1986a), capillary column gas chromatography/fourier transform infrared (GC/FT-IR) spectrometry (EPA 1986a), and high performance liquid chromatography (HPLC) (EPA 1982a). The GC/MS determination of 3,3'-DCB involves extraction into a methylene chloride or chloroform solvent, followed by separation without derivatization. Separation may be achieved on a gas chromatographic fused-silica capillary column coated with a slightly polar silicone material and detection with a mass spectrometer or electron capture detector.

For the HPLC determination of 3,3'-DCB in water, a relatively complicated procedure may be used (EPA 1982a) in which the analyte is extracted into chloroform, back-extracted with acid, neutralized and extracted with chloroform. The chloroform is exchanged to methanol and concentrated using a rotary evaporator and nitrogen blowdown, then brought to a 5 mL volume with an acetate buffer. Conditions are used that permit the separation of 3,3'-DCB compound by HPLC and measurement with an electrochemical detector, which is now currently favored over

6. ANALYTICAL METHODS

TABLE 6-1. Analytical Methods for 3,3'-Dichlorobenzidine in Biological Samples

Sample type	Extraction/cleanup	Detection	Limit of Detection	References
Animal chow	NR	GC	NR	Bowman and Rushing 1981
Fish tissue	Digest NaOH, extract benzene cleanup, concentration	GC/NPD/HCD	<20 ppb	Diachenko 1979
Urine (hamster)	Extract with benzene, heptafluorbutyryl derivative	GC/ECD	8 µg/L	Bowman and Nony 1981; Nony and Bowman 1980; Nony et al. 1980
Urine	Extract with benzene	HPLC/Spec HPLC/UV	525 µg/L	Bowman and Nony 1981; Nony and Bowman 1980; Nony et al. 1980
Urine	Extract with benzene, penta fluoropropyl derivative	GC	~1 µg/L	Bowman and Rushing 1981
Urine	NR	Spec	<1 µg/L	Roberts and Rossand 1982
Urine (for 3,3'-DCB and metabolites)	Extract with benzene, heptafluorbutyryl derivative	GC/ECD	<100 µg/L	Bowman and Nony 1981

NR = not reported; GC = gas chromatography; NPD = nitrogen-phosphorus detector; HCD = Hall conductivity detector; ECD = electron capture detector; HPLC = high performance liquid chromatography; Spec = spectrophotometry; UV = ultraviolet absorption.

spectrophotometric measurement (Trippel-Schulte et al. 1986). The method detection limit with HPLC separation and electrochemical detection is reported to be 0.13 μg/L and single operator accuracy and precision for 30 analytes of 5 different types of water samples over a spike range of 1.0 to 5.0 μg/L gave an average percent recovery of 65% and a standard deviation of 9.6% (EPA 1982a).

HPLC separation with ultraviolet absorption (UV) detection is used for the determination of 3,3'-DCB in air (NIOSH 1985). The analyte at levels in a range from 0.2 to 7 ug per sample can be collected in a silica gel collection tube for up to 100 L of air at a flow rate of 0.2 L/min. The estimated limit of detection is 0.05 μg/sample.

The most important methods for measuring 3,3'-DCB levels in air, water, food and urine are GC and HPLC procedures. For both methods, the most difficult step in the procedure is the extraction of the chemical from its matrix. While extraction of the chemical from collected air samples poses little difficulty, its extraction from a more complex matrix such as urine is more difficult. The extraction steps in themselves often result in poor recovery and have the potential for increasing assay variability. When extremely high sensitivity is required (< 1 μg/L), GC - coupled with the preparation of derivatized 3,3'-DCB is the method of choice. However, as with extractions, the derivatization process introduces another step which may decrease analytical precision. If extreme sensitivity is not required, HPLC would be preferred to GC because of the simplicity of the HPLC procedure. The two types of detectors currently used in conjunction with HPLC are ultraviolet (UV) and electrochemical (EC). EC detectors are generally 20 times more sensitive than UV detectors.

Methods for the determination of 3,3'-DCB in environmental samples are summarized in Table 6-2.

6.3 ADEQUACY OF THE DATABASE

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

6. ANALYTICAL METHODS

TABLE 6-2. Analytical Methods for 3,3'-Dichlorobenzidine in Environmental Media

Sample type	Extraction/cleanup	Detection	Limit of Detection	References
Air	Collect, extract with chloroform	HPLC GC/MS	NR	Sittig 1985
Air	Glass fiber filter, silica gel, extract	HPLC	3 $\mu g/m^3$	Verschueren 1983
Air	Glass fiber filter and silica gel, extract with triethylamine-methanol	HPLC	3 $\mu g/m^3$	Morales et al. 1981
Air	Filter, elute with triethylamine in methanol	HPLC/UV	NR	Sittig 1985
Water	Chloroform, exchange to methanol, evaporate	HPLC/ELCD	0.13 µg/L	EPA 1982a
Water	Dichloromethane, dry, evaporate	GC/MS	16.5 µg/L	EPA 1982b
Water	Dichloromethane, dry, concentrate by evaporation	GC/IDMS	50 µg/L	EPA 1984
Wastewater	NR	HPLC/UV HPLC/ELCD	<3 µg/L	Armentrout and Cutie 1980
Wastewater	Extract, convert to pentafluoropropionamides	HPLC/ELCD	0.2 pg	Kawahara et al. 1982
Wastewater	NR	HPLC	NR	Riggin et al. 1979
Soil, sediment, solid waste	Dichloromethane, dry, evaporate	GC/MS	NR	EPA 1986a
Soil, sediment, solid waste	Dichloromethane, dry,	GC/FT-IR	NR	EPA 1986b

HPLC = high performance liquid chromatography; NR = not reported; GC = gas chromatography; MS = mass spectrometry; UV = ultraviolet absorption; ELCD = electrochemical detector; IDMS = isotope dilution mass spectrometry; FT-IR = fourier transform infrared.

6. ANALYTICAL METHODS

The following discussion highlights the availability, or absence, of exposure and toxicity information applicable to human health assessment. A statement of the relevance of identified data needs is also included. In a separate effort, ATSDR, in collaboration with NTP and EPA, will prioritize data needs across chemicals that have been profiled.

6.3.1 Data Needs

Method for Determining Parent Compounds and Metabolites in Biological Materials. Methods for the determination of parent 3,3'-DCB in biological materials are adequate. Although obviously specific for the occurrence of 3,3'-DCB exposure, the sensitivity of urinary 3,3'-DCB measurement as an index of severity of 3,3'-DCB exposure needs to be established.

Methods for Biomarkers of Exposure. Adducts are reported to occur between 3,3'-DCB and nucleic acid; however, the relevance to effects, genotoxicity and carcinogenicity, are not known.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. With the exception of aquatic samples, the database for the determination of 3,3'-DCB in environmental media is adequate.

There exists an ongoing effort to develop a "Master Analytical Scheme" for organic compounds in water (Michael et al. 1988). The overall goal is to detect and quantitatively measure organic compounds at 0.1 μg/L in drinking water, 1 μg/L in surface waters, and 10 μg/L in effluent waters. Analytes are to include numerous semivolatile compounds and some compounds that are only "semi-soluble" in water, as well as volatile compounds (bp <150°C). A comprehensive review of the literature leading up to these efforts has been published (Pellizzari et al. 1985).

Studies designed to improve the determination of environmental semivolatile compounds will continue to yield refinements and improvements in the environmental determination of 3,3'-DCB. The current high level of activity in supercritical fluid extraction of solid and semisolid samples should yield improved recoveries and sensitivities for the determination of 3,3'-DCB in solid wastes and the

compound should be amenable to supercritical fluid chromatographic analysis. Immunoassay analysis Vanderlaan et al. 1988) is an area of intense current activity from which substantial advances in the determination of 3,3'-DCB in environmental samples can be anticipated.

6.3.2 Ongoing Studies

Supercritical fluid extraction/chromatography and immunoassay analysis are two areas of intense current activity from which substantial advances in the determination of 3,3'-DCB and its metabolites in biological samples can be anticipated. The two techniques are complementary in that supercritical fluid extraction is especially promising for the removal of analytes from sample material (Hawthorne 1988) and immunoassay analysis is very analyte-selective and sensitive (Vanderlaan et al. 1988).

An especially promising approach to the determination of 3,3'-DCB in biological samples is supercritical fluid extraction coupled with supercritical fluid chromatography. This combination has been described for the determination of sulfonylurea herbicides and their metabolites in complex matrices, including soil, plant materials, and a cell culture medium (McNally and Wheeler 1988). The approach described in this work should be applicable to many other toxicologically and environmentally significant analytes including 3,3'-DCB.

Thermospray techniques interfaced with mass spectrometry, with or without high performance liquid chromatographic separation, are proving useful for the determination of thermally labile compounds such as toxicant metabolites (Korfmacher et al. 1987) and should be applicable to the determination of 3,3'-DCB in biological materials (see also Betowski et al. 1987).

7. REGULATIONS AND ADVISORIES

Because of its potential to cause adverse health effects in exposed people, a number of regulations and advisory values have been established for 3,3'-DCB by various international, national and state agencies. These values are summarized in Table 7-1.

7. REGULATIONS AND ADVISORIES

TABLE 7-1. Regulations and Advisories Applicable to 3,3'-Dichlorobenzidine

Agency	Description	Value	References
	International		
IARC	Carcinogenic classification	Group 2B	IARC 1982b
	National		
Regulations			
a. Air			
OSHA	Cancer-suspect agent; Specific regulations	Stringent workplace controls, record keeping and medical surveillance	29 CFR 1910.1007
b. Water			
EPA OWRS	General permits under the National Pollutant Discharge Elimination System (NPDES)	NA[a]	40 CFR 122 Appendix D Table II
	General Pretreatment Regulations for Existing and New Sources of Pollution	NA	40 CFR 403
c. Non-specific Media			
EPA OERR	Reportable quantity	1 lb	40 CFR 302.4 EPA 1985
	Reportable quantity (proposed)	1 lb	EPA 1987a
EPA OSW	Hazardous Waste Constituent (Appendix VIII)	NA	40 CFR 261 EPA 1980c
	Ground-water Monitoring List (Appendix IX)	NA	40 CFR 264 EPA 1987b
Guidelines			
a. Air			
ACGIH	Threshold limit value (TLV) Suspected Human Carcinogen	None	ACGIH 1986
NIOSH	Recommended Exposure Limit for Occupational Exposure	Potential human carcinogen. Use 29 CFR 1910.1007.	NIOSH 1986

7. REGULATIONS AND ADVISORIES

TABLE 7-1 (continued)

Agency	Description	Value	References
b. Water			
EPA OWRS	Ambient Water Quality Criteria to Protect Human Health[b]		EPA 1980d
	Ingesting Water and Organisms		
	10^{-5}	1.03E-4 mg/L	
	10^{-6}	1.03E-5 mg/L	
	10^{-7}	1.03E-6 mg/L	
	Ingesting Organisms Only		
	10^{-5}	2.04E-4 mg/L	
	10^{-6}	2.04E-5 mg/L	
	10^{-7}	2.06E-6 mg/L	
c. Other			
EPA	Carcinogenic Classification	B2	EPA 1986c
	State Regulations and Guidelines		
State Environmental Agencies	Drinking Water Standards and Guidelines		FSTRAC 1988
	Kansas	0.21 ug/L	
	Minnesota	0.21 ug/L	
State Environmental Agencies	Acceptable Ambient Guidelines or Standards	Air Concentration	NATICH 1987
	New York	0.1 ug/m^3 (1 yr)	
	Rhode Island	0.002 ug/m^3 (annual)	
	Virginia	0 ug/m^3 (24 hr)	

[a]NA = not applicable.
[b]Because of its carcinogenic potential, the EPA-recommended value for 3,3'-DCB in ambient water is zero. However, because attainment of this level may not be possible, levels which correspond to upper-bound incremental cancer risks of 10^{-5}, 10^{-6} and 10^{-7} are estimated.

IARC = International Agency for Research on Cancer; OSHA = Occupational Safety and Health Administration; CFR = Code of Federal Regulation; EPA = Environmental Protection Agency; OWRS = Office of Water Regulations and Standards; OERR = Office of Emergency and Remedial Response; OSW = Office of Solid Waste; ACGIH = American Conference of Governmental Industrial Hygienists; NIOSH = National Institute for Occupational Safety and Health; FSTRAC = Federal-State Toxicology and Regulatory Alliance Committee; NATICH = National Air Toxics Information Clearinghouse.

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9. GLOSSARY

Acute Exposure -- Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption Coefficient (Koc) -- The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

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

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

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

Carcinogen -- A chemical capable of inducing cancer.

Ceiling value (CL) -- A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure -- Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

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

Embryotoxicity and Fetotoxicity -- Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurred. The terms, as used here, include malformations and variations, altered growth, and in utero death.

9. GLOSSARY

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

Immediately Dangerous to Life or Health (IDLH) -- The maximum environmental concentration of a contaminant from which one could escape within 30 min without any escape-impairing symptoms or irreversible health effects.

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

Immunologic Toxicity -- The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

In vitro -- Isolated from the living organism and artificially maintained, as in a test tube.

In vivo -- Occurring within the living organism.

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

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

Lethal Dose (LO) (LD_{LO}) -- The lowest dose of a chemical introduced by a route other than inhalation that is expected to have caused death in humans or animals.

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

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

9. GLOSSARY

LT50 (lethal time) -- A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

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

Minimal Risk Level -- An estimate of daily human exposure to a chemical that is likely to be without an appreciable risk of deleterious effects (noncancerous) over a specified duration of exposure.

Mutagen -- A substance that causes mutations. A mutation is a change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

Neurotoxicity -- The occurrence of adverse effects on the nervous system following exposure to a chemical.

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

Octanol-Water Partition Coefficient (Kow) -- The equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution.

Permissible Exposure Limit (PEL) -- An allowable exposure level in workplace air averaged over an 8-h shift.

q^1* -- The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q^1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually $\mu g/L$ for water, mg/kg/day for food, and $\mu g/m^3$ for air).

Reference Dose (RfD) -- An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the NOAEL (from animal and human studies) by a consistent application of

uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ) -- The quantity of a hazardous substance that is considered reportable under CERCLA. Reportable quantities are: (1) 1 lb or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Sect. 311 of the Clean Water Act. Quantities are measured over a 24-h period.

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

Short-Term Exposure Limit (STEL) -- The maximum concentration to which workers can be exposed for up to 15 min continually. No more than four excursions are allowed per day, and there must be at least 60 min between exposure periods. The daily TLV-TWA may not be exceeded.

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

TD50 (toxic dose) -- A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

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

Threshold Limit Value (TLV) -- A concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a TWA, as a STEL, or as a CL.

Time-weighted Average (TWA) -- An allowable exposure concentration averaged over a normal 8-h workday or 40-h workweek.

9. GLOSSARY

Uncertainty Factor (UF) -- A factor used in operationally deriving the RfD from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of humans, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

APPENDIX: PEER REVIEW

A peer review panel was assembled for 3,3'-DCB. The panel consisted of the following members: Dr. Paul Mushak, Private Consultant, Durham, North Carolina; Dr. David Jollow, Professor, Medical University of South Carolina; Dr. T.J. Kneip, Professor, New York University Medical Center. These experts collectively have knowledge of 3,3'-DCB's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in the Superfund Amendments and Reauthorization Act of 1986, Section 110.

A joint panel of scientists from ATSDR and EPA has reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with the Agency for Toxic Substances and Disease Registry.

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