TOXICOLOGICAL PROFILE FOR 3,3'-DICHLOROBENZIDINE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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UPDATE STATEMENT

A Toxicological Profile for 3,3'-Dichlorobenzidine was released in September 1997. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary, but no less than once every three years. For information regarding the update status of previously released profiles, contact ATSDR at:

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FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but is 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.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning 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 are identified by ATSDR and EPA.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance 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 that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

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.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

Jeffrey P. Koplan, M.D., M.P.H.
Administrator
Agency for Toxic Substances and
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The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on November 17, 1997 (62 FR 61332). For prior versions of the list of substances, see *Federal Register* notices dated April 29, 1996 (61 FR 18744); April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17,199O (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); and February 28, 1994 (59 FR 9486). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement:

The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Health Effects:

Specific health effects of a given hazardous compound are reported by route *of exposure,* by *type of health effect* (death, systemic, immunologic, reproductive), and by *length of exposure* (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.

NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:

Section 1.6 How Can (Chemical X) Affect Children?
Section 1.7 How Can Families Reduce the Risk of Exposure to (Chemical X)?
Section 2.6 Children's Susceptibility
Section 5.6 Exposures of Children

Other Sections of Interest:

Section 2.7 Biomarkers of Exposure and Effect
Section 2.10 Methods for Reducing Toxic Effects

ATSDR Information Center

Phone: l-800-447-1544 (to be replaced by 1-888-42-ATSDR in 1999)
or 404-639-6357 ***Fax:*** *404-639-6359*
E-mail: atsdric@,cdc.nov ***Internet:*** http://atsdrl.atsdr.cdc.nov:8080

The following additional material can be ordered through the ATSDR Information Center:

Case Studies in Environmental Medicine: Taking an Exposure History-The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide Toxicity;* and numerous chemical-specific case studies.

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III-*MedicalManagement Guidelines for Acute Chemical Exposures*-is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets ('ToxFAQs) provide answers to frequently asked questions about toxic substances.

Other Agencies and Organizations

The National Centerfor Environmental Health (NCEH)
focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. *Contact:* NCEH, Mailstop F-29,4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

The National Institute for Occupational Safety and Health (NIOSH)
conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. *Contact:* NIOSH, 200 Independence Avenue, SW, Washington, DC 2020 1 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19,4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

The National Institute of Environmental Health Sciences (NIEHS)
is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. *Contact:* NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

Referrals

The Association of Occupational and Environmental Clinics (AOEC)
has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. *Contact:* AOEC, 10 10 Vermont Avenue, NW, #5 13, Washington, DC 20005 l Phone: 202-347-4976 l FAX: 202- 347-4950 • e-mail: aoec@,dgs.dnsys.com • AOEC Clinic Director: http://occ-envmed. mc.duke.edu/oem/aoec.htm.

The American College of Occupational and Environmental Medicine (ACOEM)
is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. *Contact:* ACOEM, 55 West Seegers Road, Arlington Heights, IL 60005 • Phone: 847-228-6850 • FAX: 847-228-1856.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2 . Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific minimal risk levels (MRLs), reviews the health effects database of each profile: and makes recommendations for derivation of MRLs.

3 . Data Needs Review. The Research Implementation Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

PEER REVIEW

A peer review panel was assembled for 3,3'-dichlorobenzidine. The panel consisted of the following members :

1. Dr. Herbert Cornish, Private Consultant, 830 W. Clark Rd., Ypsilanti, MI 48 198;
2. Dr. Arthur Gregory, Private Consultant, 1 Gregory Lane, Luray, VA 22835;
3. Dr. Philip Leber, Private Consultant, 1344 Jefferson Ave., Akron, OH 443 13; and
4. Dr. Robert Rubin, Johns Hopkins School of Public Health, Environmental Health Sciences, Baltimore, MD 21205.

These experts collectively have knowledge of 3,3'-dichlorobenzidine'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 Section 104(1)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have 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 its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

CONTENTS

FOREWORD v

QUICK REFERENCE FOR HEALTH CARE PROVIDERS vii

CONTRIBUTORS ix

PEER REVIEW xi

LIST OF FIGURES xvii

LIST OF TABLES xix

1. PUBLIC HEALTH STATEMENT 1
   - 1.1 WHAT IS 3,3'-DICHLOROBENZIDINE? 1
   - 1.2 WHAT HAPPENS TO 3,3'-DICHLOROBENZIDINE WHEN IT ENTERS THE ENVIRONMENT? 2
   - 1.3 HOW MIGHT I BE EXPOSED TO 3,3'-DICHLOROBENZIDINE? 2
   - 1.4 HOW CAN 3,3'-DICHLOROBENZIDINE ENTER AND LEAVE MY BODY? 3
   - 1.5 HOW CAN 3,3'-DICHLOROBENZIDINE AFFECT MY HEALTH? 3
   - 1.6 HOW CAN 3,3'-DICHLOROBENZIDINE AFFECT CHILDREN? 5
   - 1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO 3,3'-DICHLOROBENZIDINE? 6
   - 1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO 3,3'-DICHLOROBENZIDINE? 7
   - 1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH? 7
   - 1.10 WHERE CAN I GET MORE INFORMATION? 9

2. HEALTH EFFECTS 11
   - 2.1 INTRODUCTION 11
   - 2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE 11
     - 2.2.1 Inhalation Exposure 12
       - 2.2.1.1 Death 13
       - 2.2.1.2 Systemic Effects 13
       - 2.2.1.3 Immunological and Lymphoreticular Effects 14
       - 2.2.1.4 Neurological Effects 14
       - 2.2.1.5 Reproductive Effects 14
       - 2.2.1.6 Developmental Effects 14
       - 2.2.1.7 Genotoxic Effects 14
       - 2.2.1.8 Cancer 15
     - 2.2.2 Oral Exposure 17
       - 2.2.2.1 Death 17
       - 2.2.2.2 Systemic Effects 24
       - 2.2.2.3 Immunological and Lymphoreticular Effects 25
       - 2.2.2.4 Neurological Effects 25
       - 2.2.2.5 Reproductive Effects 26

2.2.2.6 Developmental Effects 26
2.2.2.7 Genotoxic Effects 26
2.2.2.8 Cancer 27
2.2.3 Dermal Exposure 29
2.2.3.1 Death 29
2.2.3.2 Systemic Effects 30
2.2.3.3 Immunological and Lymphoreticular Effects 32
2.2.3.4 Neurological Effects 32
2.2.3.5 Reproductive Effects 32
2.2.3.6 Developmental Effects 32
2.2.3.7 Genotoxic Effects 32
2.2.3.8 Cancer 32
2.3 TOXICOKINETICS 34
2.3.1 Absorption 34
2.3.1.1 Inhalation Exposure 34
2.3.1.2 Oral Exposure 35
2.3.1.3 Dermal Exposure 35
2.3.2 Distribution 35
2.3.2.1 Inhalation Exposure 36
2.3.2.2 Oral Exposure 36
2.3.2.3 Dermal Exposure 37
2.3.3 Metabolism 37
2.3.4 Elimination and Excretion 39
2.3.4.1 Inhalation Exposure 39
2.3.4.2 Oral Exposure 39
2.3.4.3 Dermal Exposure 40
2.3.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models 40
2.4 MECHANISMS OF ACTION 43
2.4.1 Pharmacokinetic Mechanisms 43
2.4.2 Mechanisms of Toxicity 44
2.4.3 Animal-to-Human Extrapolations 45
2.5 RELEVANCE TO PUBLIC HEALTH 46
2.6 CHILDREN'S SUSCEPTIBILITY 59
2.7 BIOMARKERS OF EXPOSURE AND EFFECT 62
2.7.1 Biomarkers Used to Identify or Quantify Exposure to 3,3'-Dichlorobenzidine 63
2.7.2 Biomarkers Used to Characterize Effects Caused by 3,3'-Dichlorobenzidine 64
2.8 INTERACTIONS WITH OTHER CHEMICALS 64
2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE 65
2.10 METHODS FOR REDUCING TOXIC EFFECTS 65
2.10.1 Reducing Peak Absorption Following Exposure 65
2.10.2 Reducing Body Burden 66
2.10.3 Interfering with the Mechanism of Action for Toxic Effects 66
2.11 ADEQUACY OF THE DATABASE 66
2.11.1 Existing Information on Health Effects of 3,3'-Dichlorobenzidine 67
2.11.2 Identification of Data Needs 69
2.11.3 Ongoing Studies 75

3. CHEMICAL AND PHYSICAL INFORMATION 77
3.1 CHEMICAL IDENTITY 77
3.2 PHYSICAL AND CHEMICAL PROPERTIES 77

4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL 81
4.1 PRODUCTION 81
4.2 IMPORT/EXPORT 81
4.3 USE 83
4.4 DISPOSAL 83

5. POTENTIAL FOR HUMAN EXPOSURE 85
5.1 OVERVIEW 85
5.2 RELEASES TO THE ENVIRONMENT 87
5.2.1 Air 89
5.2.2 Water 91
5.2.3 Soil 91
5.3 ENVIRONMENTAL FATE 92
5.3.1 Transport and Partitioning 92
5.3.2 Transformation and Degradation 94
5.3.2.1 Air 94
5.3.2.2 Water 95
5.3.2.3 Sediment and Soil 96
5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT 98
5.4.1 Air 98
5.4.2 Water 99
5.4.3 Sediment and Soil 99
5.4.4 Other Environmental Media 99
5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE 100
5.6 EXPOSURES OF CHILDREN 101
5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES 102
5.8 ADEQUACY OF THE DATABASE 103
5.8.1 Identification of Data Needs 103
5.8.2 Ongoing Studies 106

6. ANALYTICAL METHODS 107
6.1 BIOLOGICAL SAMPLES 107
6.2 ENVIRONMENTAL SAMPLES 110
6.3 ADEQUACY OF THE DATABASE 113
6.3.1 Identification of Data Needs 113
6.3.2 Ongoing Studies 114

7. REGULATIONS AND ADVISORIES 115

8. REFERENCES 123

9. GLOSSARY 139

APPENDICES

A. ATSDR MINIMAL RISK LEVEL AND WORKSHEETS A-1

B. USER'S GUIDE B-1

C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS C-1

LIST OF FIGURES

2-1 Levels of Significant Exposure to 3,3'-Dichlorobenzidine—Oral 22

2-2 Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance 42

2-3 Existing Information on Health Effects of 3,3'-Dichlorobenzidine 68

5-1 Frequency of NPL Sites with 3,3'-Dichlorobenzidine Contamination 90

LIST OF TABLES

2-1 Levels of Significant Exposure to 3,3'-Dichlorobenzidine—Oral 18

2-2 Levels of Significant Exposure to 3,3'-Dichlorobenzidine—Dermal 31

2-3 Genotoxicity of 3,3'-Dichlorobenzidine *In Vivo* 53

2-4 Genotoxicity of 3,3'-Dichlorobenzidine *In Vitro* 54

3-1 Chemical Identity of 3,3'-Dichlorobenzidine 78

3-2 Physical and Chemical Properties of 3,3'-Dichlorobenzidine 79

4-1 Facilities That Manufacture or Process 3,3'-Dichlorobenzidine 82

5-1 Releases to the Environment from Facilities That Manufacture or Process 3,3'-Dichlorobenzidine 88

6-1 Analytical Methods for Determining 3,3'-Dichlorobenzidine and Metabolites in Biological Samples 108

6-2 Analytical Methods for Determining 3,3'-Dichlorobenzidine in Environmental Samples 111

7-1 Regulations and Guidelines Applicable to 3,3'-Dichlorobenzidine 118

1. PUBLIC HEALTH STATEMENT

This public health statement tells you about 3,3'-dichlorobenzidine and the effects of exposure.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites make up the National Priorities List (NPL) and are the sites targeted for long-term federal cleanup activities. 3,3'-Dichlorobenzidine has been found in at least 32 of the 1,467 current or former NPL sites. However, the total number of NPL sites evaluated for this substance is not known. As more sites are evaluated, the sites at which 3,3'-dichlorobenzidine is found may increase. This information is important because exposure to this substance may harm you and because these sites may be sources of exposure.

When a substance is released from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. This release does not always lead to exposure. You are exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance or by skin contact.

If you are exposed to 3,3'-dichlorobenzidine, many factors determine whether you'll be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. You must also consider the other chemicals you're exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

1 .l WHAT IS 3,3'-DICHLOROBENZIDINE?

3,3'-Dichlorobenzidine is a gray-to-purple colored crystalline solid. It changes from a solid to a gas very slowly. 3,3'-Dichlorobenzidine salt, the major form in actual use, is a stable, off-white colored crystalline solid that does not evaporate. Neither 3,3'-dichlorobenzidine nor its salt occur naturally in the environment. They are manufactured for use in the production of pigments for printing inks, textiles, plastics and enamels, paint, leather, and rubber. Whether 3,3'-dichlorobenzidine or the salt is present as such depends on the acidity of the soil or water as well as other

factors. In most environmental samples, such as water and soils, 3,3'-dichlorobenzidine would be expected to exist in the free amino form, not as the salt. For more information, see Chapters 3 and 4.

1.2 WHAT HAPPENS TO 3,3'-DICHLOROBENZIDINE WHEN IT ENTERS THE ENVIRONMENT?

3,3'-Dichlorobenzidine breaks down rapidly when exposed to natural sunlight. In air and sunshine, it is estimated that half of the chemical breaks down within 9.7 hours. In water exposed to natural sunlight, 3,3'-dichlorobenzidine is expected to break down rapidly, with half being removed in approximately 90 seconds. In soil, where no sunlight is present, the compound may last for several months. Under certain conditions, 3,3'-dichlorobenzidine can break down in soil to form another compound, benzidine, which is toxic. For more information, see Chapter 5.

1.3 HOW MIGHT I BE EXPOSED TO 3,3'-DICHLOROBENZIDINE?

3,3'-Dichlorobenzidine is used to make pigments (substances used to give color to something, for example, paint). You are most likely to be exposed to 3,3'-dichlorobenzidine if you work inside plants where the chemical is manufactured or used. However, employers have limited workers' exposure to the chemical by using closed systems for processing as well as other methods for reducing its concentration in the air to very low levels and by requiring workers to wear protective clothing and use special equipment. If you were exposed in such a workplace, it would probably be by breathing in the dust or by getting the chemical on your skin. Careless handling or accidental spillage of the chemical could result in exposure to potentially hazardous levels of 3,3'-dichlorobenzidine. People may be exposed to the chemical if they live or work near land where plant wastes have been stored or buried, or close to lakes, streams, or rivers near where plants discharge process water or store wastes. Most people do not live near a source of the chemical. The Canadian government has published calculations that show that exposure of the Canadian general population to 3,3'-dichlorobenzidine in air, soil, or water is extremely low. If you do live in areas near a source of the chemical (such as a hazardous waste site that contains

dye or pigment manufacturing wastes), some exposure could occur if you or a child accidentally or purposely ingested small amounts of contaminated soil, drank contaminated water, or ate fish caught in waters near the source. However, studies of water and fish taken from locations near dye-manufacturing plants did not find the chemical.

3,3'-Dichlorobenzidine has no agricultural or food chemical uses, so exposure to it by eating contaminated food is not likely. More information about the presence of 3,3'-dichlorobenzidine in our environment and how it disappears by being broken down by other chemicals and processes can be found in Chapter 5.

1.4 HOW CAN 3,3'-DICHLOROBENZIDINE ENTER AND LEAVE MY BODY?

In the workplace, 3,3'-dichlorobenzidine may enter the body when workers breathe dust contaminated by 3,3'-dichlorobenzidine and through skin contact. You are not likely to be exposed to 3,3'-dichlorobenzidine unless you drink water or eat dirt contaminated with 3,3'-dichlorobenzidine in the vicinity of a hazardous waste site where 3,3'dichlorobenzidine has been stored and leakage has occurred. When 3,3'-dichlorobenzidine 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 urine and to a lesser extent in feces, within 72 hours after exposure. More information can be found in Chapter 2.

1.5 HOW CAN 3,3'-DICHLOROBENZIDINE AFFECT MY HEALTH?

Some workers exposed to the salt form of 3,3'-dichlorobenzidine complained of sore throat, respiratory infections, stomach upset, headache, dizziness, caustic burns, and dermatitis (an inflammation of the skin). However, with the exception of dermatitis, it is not certain that 3,3'-dichlorobenzidine causes these health effects because the workers were also exposed to other chemicals at the same time. There is no evidence that 3,3'-dichlorobenzidine affects the nervous system, the ability to fight disease, or the ability of people to have children.

1. PUBLIC HEALTH STATEMENT

To protect the public from the harmful effects of toxic chemicals and to find ways to treat people who have been harmed, scientists use many tests.

One way to see if a chemical will hurt people is to learn how the chemical is absorbed, used, and released by the body; for some chemicals, animal testing may be necessary. Animal testing may also be used to identify health effects such as cancer or birth defects. Without laboratory animals, scientists would lose a basic method to get information needed to make wise decisions to protect public health. Scientists have the responsibility to treat research animals with care and compassion. Laws today protect the welfare of research animals, and scientists must comply with strict animal care guidelines.

Death has occurred in laboratory animals that ate very high levels of 3,3'-dichlorobenzidine mixed in their food for short periods of time. Laboratory animals exposed to moderate levels of 3,3'-dichlorobenzidine mixed with food for a long time suffered mild injury to the liver.

Studies show that 3,3'-dichlorobenzidine caused cancer of the liver, skin, breast, bladder, and tissues that form blood (leukemia), and other sites in laboratory animals that ate 3,3'-dichlorobenzidine in their food. There is no evidence that 3,3'-dichlorobenzidine has caused cancer in people who worked with it or who were exposed to it unknowingly or by accident for a short or long time. However, because of the many types of cancer that 3,3'-dichlorobenzidine has caused in different tissues of many types of laboratory animals, 3,3'-dichlorobenzidine should be thought of as probably capable of causing human cancer if exposure to the chemical is sufficiently high.

The Environmental Protection Agency (EPA) has determined that 3,3'-dichlorobenzidine is a "probable human carcinogen." The U.S. Department of Health and Human Services (DHHS) has determined that 3,3'-dichlorobenzidine and its salt may reasonably be expected to be cancercausing substances (carcinogens). The International Agency for Research on Cancer (IARC) has determined that 3,3'-dichlorobenzidine is possibly carcinogenic to humans. More information can be found in Chapter 2.

1. PUBLIC HEALTH STATEMENT

1.6 HOW CAN 3,3'-DICHLOROBENZIDINE AFFECT CHILDREN?

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on children resulting from exposures of the parents are also considered.

Children might be exposed to 3,3'-dichlorobenzidine if they eat small amounts of soil contaminated with 3,3'-dichlorobenzidine. However, studies suggest that it is very difficult to release 3,3'-dichlorobenzidine once it becomes attached to soil. Exposure via contaminated soil may occur if they live in an area near a source of the chemical (such as a hazardous waste site that contains 3,3'-dichlorobenzidine). Children can also be exposed if the parents work at chemical facilities where 3,3'-dichlorobenzidine is handled and bring home contaminated clothing or tools or if they do not shower before coming home. There are no known unique exposure pathways for children.

There have been no studies of health effects in children exposed to 3,3'-dichlorobenzidine. We have no information on whether 3,3'-dichlorobenzidine causes birth defects in children. It is unknown whether birth defects would occur in the offspring of pregnant animals that breathed or eaten 3,3'-dichlorobenzidine, or had it on their skin. In studies in which pregnant mice were injected with high amounts of 3,3'-dichlorobenzidine under the skin, the kidneys of their babies did not develop properly and some babies developed renal tumors. However, it is highly unlikely that humans will encounter such exposure conditions.

There is no information to determine whether children are different in their sensitivity to the health effects of 3,3'-dichlorobenzidine from adults. There is indirect evidence that 3,3'-dichlorobenzidine or its breakdown products can cross the placenta, but we do not know for certain whether it can be transferred to the young via the mother's breast milk. Sometimes when children have been exposed to chemicals before they are born, the chemical or its breakdown products can be found in amniotic fluid, meconium, cord blood, or neonatal blood; however, no information

about such measurements was found for 3,3'-dichlorobenzidine. More information regarding children's health and 3,3'-dichlorobenzidine can be found in Section 2.6.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO 3,3'-DICHLOROBENZIDINE?

If your doctor finds that you have been exposed to significant amounts of 3,3'-dichlorobenzidine, ask your doctor if children may also be exposed. When necessary your doctor may need to ask your state Department of Public Health to investigate.

3,3'-Dichlorobenzidine has no agricultural or food chemical uses, so exposure to it by eating contaminated food is not likely. It is sometimes possible to carry 3,3'-dichlorobenzidine from work on your clothing, skin, hair, tools, or other objects removed from the workplace. This has happened in factories that produce 3,3'-dichlorobenzidine. In this way, you may contaminate your car, home, or other locations outside work where children might be exposed to 3,3'-dichlorobenzidine. You should know about this possibility if you work with 3,3'-dichlorobenzidine.

Your occupational health and safety officer at work can and should tell you whether chemicals you work with are dangerous and likely to be carried home'on your clothes, body, or tools. Ask if you should shower and change clothes before you leave work, store your street clothes in a separate area of the workplace, or launder your work clothes at home separately from other clothes. The Occupational Safety and Health Administration (OSHA) requires Material Safety Data Sheets (MSDSs) for many chemicals used at your place of work. MSDS information should include chemical names and hazardous ingredients, and important information such as fire and explosion data, potential health effects, how you get the chemical(s) in your body, how to properly handle the materials, and what to do in the case of emergencies. Your employer is legally responsible for providing a safe workplace and should freely answer your questions about hazardous chemicals. U.S. OSHA or your state OSHA-approved occupational safety and health program can answer any further questions and help your employer identify and correct problems with hazardous substances. OSHA or your state OSHA-approved occupational safety and health program will listen to your formal complaints about workplace health hazards and inspect your

workplace when necessary. Employees have a right to seek safety and health on the job without fear of punishment. More information regarding exposure to 3,3'-dichlorobenzidine can be found in Sections 5.5, 5.6, and 5.7.

1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO 3,3'-DICHLOROBENZIDINE?

Exposure to 3,3'-dichlorobenzidine can be determined by finding the chemical or its metabolites in urine. The test is not commonly available to the general population, but it is available to workers who may be exposed to potentially hazardous levels of the chemical in the workplace (for example, by careless handling or accidental spills). The test is accurate and provides evidence that exposure has occurred. However, since 3,3'-dichlorobenzidine does not remain long in the body, the test must be performed very soon after the possible exposure. Also, measured urine levels of 3,3'-dichlorobenzidine or its metabolites do not tell you whether it will affect your health. More information can be found in Chapter 6.

1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

Federal agencies that develop regulations for toxic substances include the Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA). Recommendations provide valuable guidelines to protect public health but cannot be enforced by law. Federal organizations that develop recommendations for toxic substances include the Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH).

Regulations and recommendations can be expressed in not-to-exceed levels in air, water, soil, or food that are usually based on levels that affect animals; then they are adjusted to help protect people. Sometimes these not-to-exceed levels differ among federal organizations because of different exposure times (an 8-hour workday or a 24-hour day), the use of different animal studies, or other factors.

1. PUBLIC HEALTH STATEMENT

Recommendations and regulations are also periodically updated as more information becomes available. For the most current information, check with the federal agency or organization that provides it. Some regulations and recommendations for 3,3'-dichlorobenzidine include the following:

EPA has determined that 3,3'-dichlorobenzidine is a "probable human carcinogen" and has placed several limits on the chemical in the environment to protect human health. Under the Clean Water Act of 1977, EPA controls discharges of 3,3'-dichlorobenzidine to industrial waste waters. The agency has listed 3,3'-dichlorobenzidine as a hazardous waste and requires that any spill of one pound or more be reported to the National Response Center.

Although the FDA has classified 3,3'-dichlorobenzidine as a carcinogen, no regulatory guidelines have been enacted. The FDA has concluded that the food supply is not in danger from 3,3'-dichlorobenzidine.

3,3'-Dichlorobenzidine is one of a number of compounds regulated by OSHA. To control exposures to 3,3'-dichlorobenzidine in workplace air and to protect the health of workers, OSHA's regulatory standards provide strict guidelines for handling, using, and storing the compound. They also include the requirements for 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'-dichlorobenzidine at potentially hazardous levels.

NIOSH considers 3,3'-dichlorobenzidine a "potential occupational carcinogen" and recommends workplace practices and controls to reduce exposures to the lowest possible level. NIOSH defines potential occupational carcinogens as substances which may cause an increased incidence of benign and/or malignant neoplasm, or a substantial decrease in the latency period between exposure and onset of neoplasms in humans.

1. PUBLIC HEALTH STATEMENT

1.10 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department or:

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

* Information line and technical assistance

Phone: l-800-447- 1544
Fax: (404) 639-6359

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses resulting from exposure to hazardous substances.

* To order toxicological profiles, contact:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22 16 1
Phone: (800) 553-6847 or (703) 487-4650

2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of 3,3'-dichlorobenzidine. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure-inhalation, oral, and dermal; and then by health effect--death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observedadverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The

distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

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

2.2.1 Inhalation Exposure

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

2. HEALTH EFFECTS

2.2.1.1 Death

No studies were located regarding lethal effects in humans or animals after inhalation exposure to 3,3'-dichlorobenzidine. No fatalities were observed in rats observed for 14 days following a l-hour exposure to an unspecified concentration of 3,3'-dichlorobenzidine dihydrochloride dust (Gerarde and Gerarde 1974). No deaths were reported in male rats exposed to 23,700 mg/m^3 3,3'-dichlorobenzidine base (dust) for 2 hours per day for 7 days (Gerarde and Gerarde 1974).

2.2.1.2 Systemic Effects

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

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

No adverse health effects were observed in male rats exposed by inhalation to 3,3'- dichlorobenzidine free base (23,700 mg/m^3) 2 hours per day for 7 days (Gerarde and Gerarde 1974). In another study, 10 rats were exposed to an unspecified concentration of 3,3'-dichlorobenzidine dihydrochloride dust particles for 1 hour and then observed for 14 days. Slight-to-moderate pulmonary congestion and one pulmonary abscess were observed upon necropsy (Gerarde and Gerarde 1974). The effects observed in the study using the ionized (hydrochloride) form of 3,3'-dichlorobenzidine may have been due to the irritative properties of hydrochloric acid released from the salt in combination with particulate toxicity.

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

2. HEALTH EFFECTS

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

2.2.1.3 Immunological and Lymphoreticular Effects

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

2.2.1.4 Neurological Effects

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

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

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

2.2.1.5 Reproductive Effects

2.2.1.6 Developmental Effects

2.2.1.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

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

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

2. HEALTH EFFECTS

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

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

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

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

2. HEALTH EFFECTS

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

2.2.2 Oral Exposure

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

2.2.2.1 Death

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

In rats, the acute-duration oral LD_{50} (lethal dose, 50% kill) for 3,3'-dichlorobenzidine free base administered in pure olive oil was estimated to be 7,070 mg/kg, whereas the LD_{50} for a 20% suspension of the dihydrochloride salt in corn oil was 3,820 mg/kg (Gerarde and Gerarde 1974). The cause of death was not discussed. Given this high LD_{50} acute lethality in humans following oral exposure is unlikely. Both oral LD_{50} values for 3,3'-dichlorobenzidine are shown in Table 2-1 and plotted in Figure 2-l.

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

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

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

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

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

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
	Cancer							
8	Rat (Rappolovskil)	12 mo 6 d/wk (F)				120	(tumors in Zymbal gland, skin, mammary gland, ileum, bladder, hemopoetic, connective tissue, salivary gland, liver, thyroid)	Pliss 1959
9	Rat (Sprague-Dawley)	16 mo ad lib (F)				70 M	(CEL: malignant mammary gland adeno- carcinomas in 7/44; Zymbal gland squamous cell carcinomas in 8/44; granulocytic leukemia in 9/44)	Stula et al. 1975
						80 F	(CEL: malignant mammary gland adenocarcinomas in 26/44 females)	
10	Hamster (Golden	NS (F)				300	(transitional cell bladder carcinomas, liver-cell and cholangiomatous tumors)	Sellakumar et al. 1969

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

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

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

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

Figure 2-1. Levels of Significant Exposure to 3,3-Dichlorobenzidine - Oral

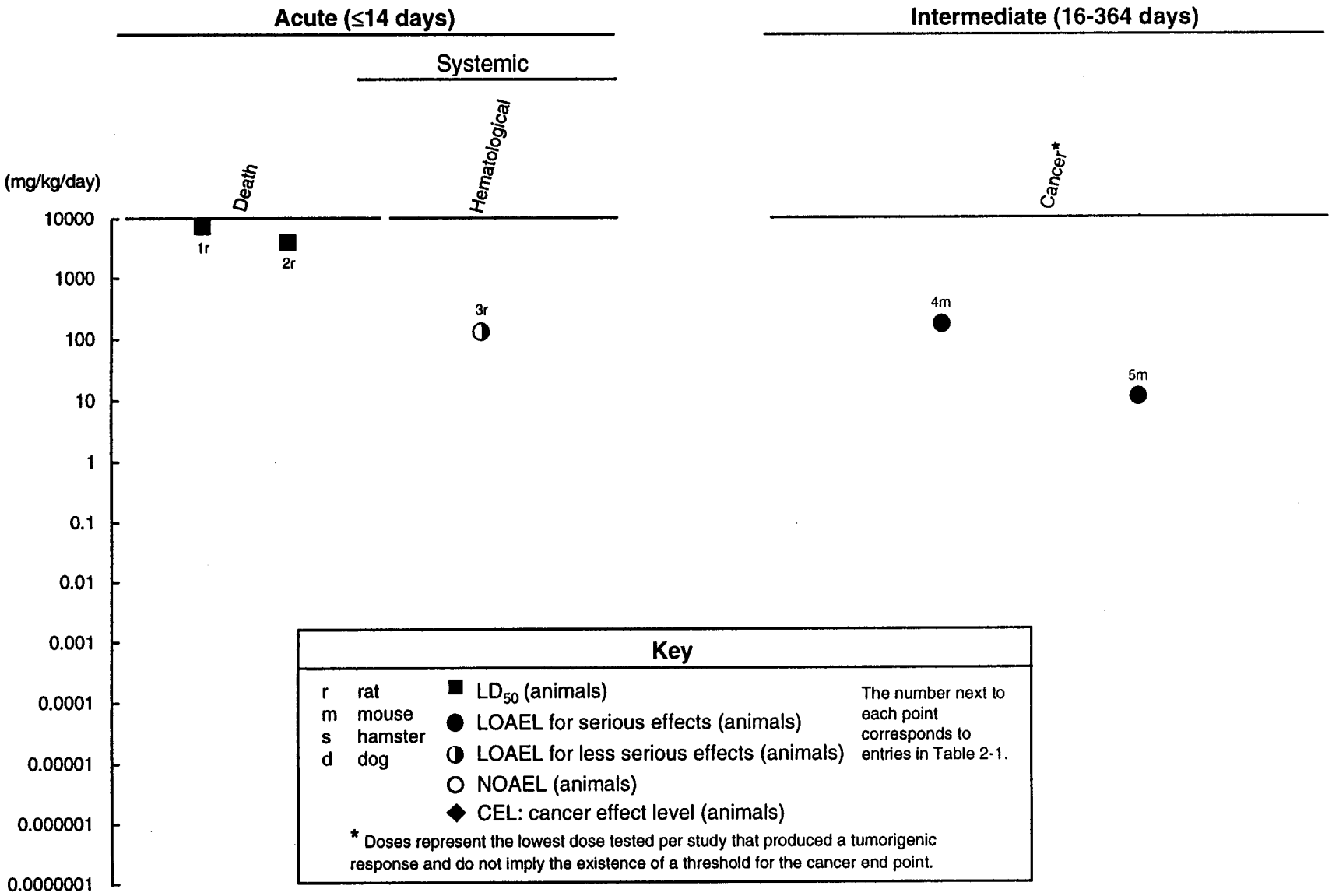
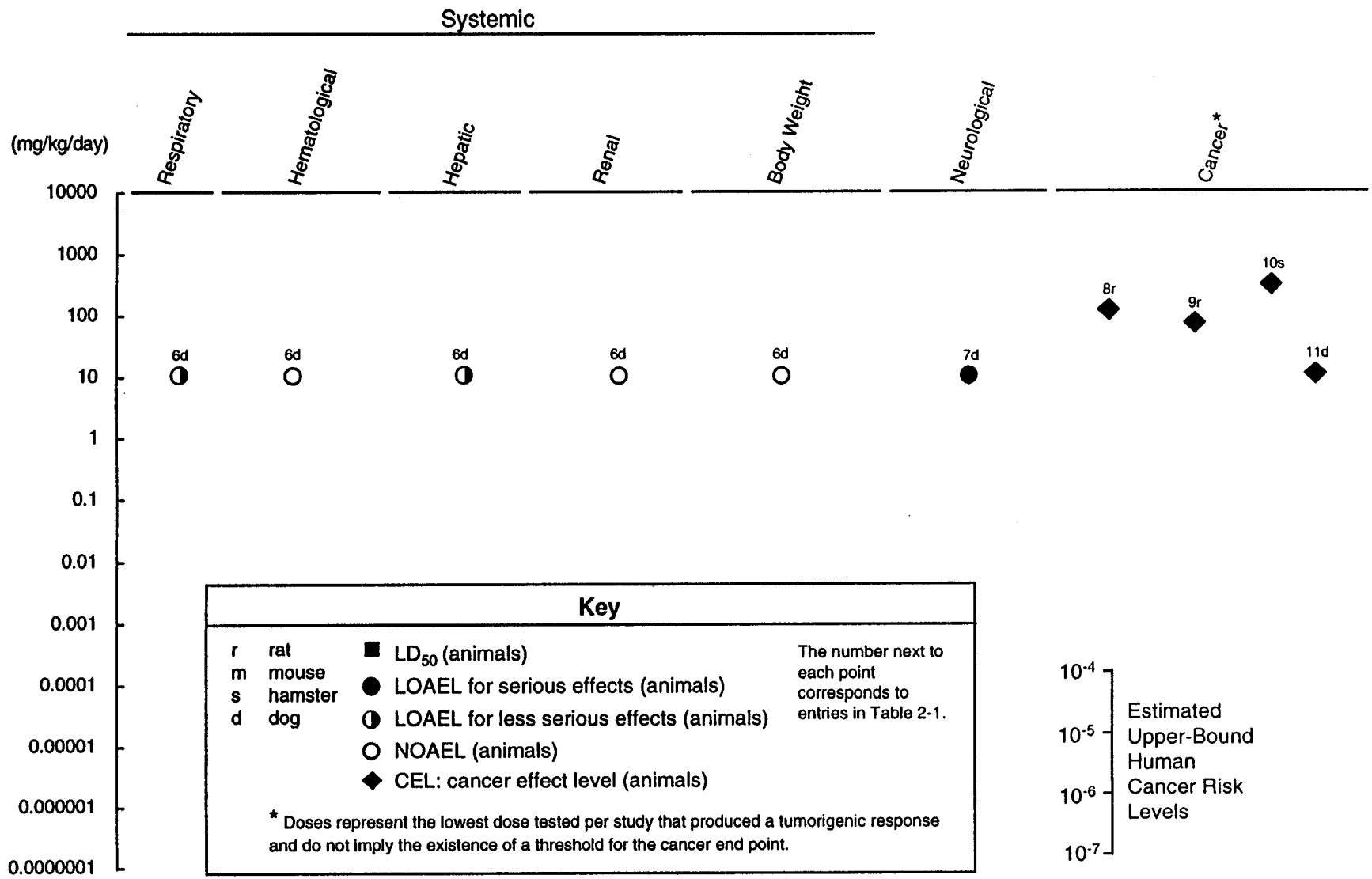


Figure 2-1. Levels of Significant Exposure to 3,3-Dichlorobenzidine - Oral (cont.)

Chronic (≥365 days)



2.2.2.2 Systemic Effects

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

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

Respiratory Effects. Dyspnea was observed in 1 of 6 female dogs exposed to 10.4 mg/kg/day 3,3'-dichlorobenzidine for 6.6 years, which probably resulted as a secondary effect of liver disease, that this dog was experiencing. No respiratory effects were observed in any other dogs, including controls (Stula et al. 1978).

Hematological Effects. Although hematological effects may not be sensitive indicators for 3,3'-dichlorobenzidine toxicity, hemoglobin adducts have been detected in female Wistar rats orally administered single 127 or 253 mg/kg doses of 3,3'-dichlorobenzidine (Birner et al. 1990) or with repeated doses between 0.3 and 5.8 mg/kg/day (Joppich-Kuhn et al. 1997). It was suggested that metabolically formed nitroso derivatives and the formation of a sulfinic acid amide with cysteine residues in hemoglobin may be the mechanism of adduct formation (Birner et al. 1990). Hydrolysis yielded mainly 3,3'-dichlorobenzidine; N-acetylated 3,3'-dichlorobenzidine was also detected. The more recent study found that adduct formation was dose-related (Joppich-Kuhn et al. 1997). It was further observed that at low doses of 3,3'-dichlorobenzidine, N-acetyl-3,3'-dichlorobenzidine adducts and 3,3'-dichlorobenzidine adducts were formed at similar levels, but at the highest dose level tested (5.8 mg/kg/day) the dichlorobenzidine adduct was predominant, suggesting saturation of the acetylation pathway at high dose (Joppich-Kuhn et al. 1997). While hemoglobin adduct formation does not imply altered or abnormal hemoglobin function, adduct formation may be a suitable biomarker of human exposure to 3,3'-dichlorobenzidine (see Section 2.7). Hematological variables (erythrocyte count, hemoglobin concentration, hematocrit, and leucocyte count) were found to be normal in dogs exposed to 10.4 mg/kg/day 3,3'-dichlorobenzidine for 7 years (Stula et al. 1978).

Hepatic Effects. Limited animal evidence suggests that chronic-duration oral exposure to 3,3'-dichlorobenzidine results in mild-to-moderate liver injury. Six female dogs exposed to 3,3'-dichlorobenzidine (10.4 mg/kg/day) all had modestly elevated plasma glutamic-pyruvic transaminase (GPT) during the first 3 years of a 7-year treatment period (Stula et al. 1978). Thereafter, GPT levels returned to normal in three of the experimental animals, two remained elevated for the duration of the study. Elevated GPT levels may have been due to the test chemical that caused chronic hepatic injury to these dogs that ultimately led to development of liver tumors. One of the six dogs, sacrificed after 42 months of the test, showed a marked fatty change in the liver. It should be noted that the study is limited by use of one dose level, precluding dose-response evaluations. It should be mentioned, however, that none of the six control dogs exhibited adverse liver effects.

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

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

2.2.2.3 Immunological and Lymphoreticular Effects

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

2.2.2.4 Neurological Effects

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

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

at 42 months revealed slight neuronal degeneration; although the specific location was not indicated, histological examination was performed on the brain and spinal cord. No neurological effects were observed in any other dogs, including controls. This LOAEL value for neurological effect for oral exposure to 3,3'-dichlorobenzidine is shown in Table 2- 1 and plotted in Figure 2- 1.

2.2.2.5 Reproductive Effects

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

2.2.2.6 Developmental Effects

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

2.2.2.7 Genotoxic Effects

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

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

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

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

2.2.2.8 Cancer

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

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

No bladder carcinomas were observed in rats exposed to 0.03% 3,3'-dichlorobenzidine in the diet (27 mg/kg/day) for 4 or 40 weeks (Ito et al. 1983), nor were any mammary tumors observed in rats administered approximately 49 mg 3,3'-dichlorobenzidine dihydrochloride/kg/day by gavage once every 3 days over a 30-day period and sacrificed 8 months later (Griswold et al. 1968).

2. HEALTH EFFECTS

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

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

In a subsequent study by this investigator, hepatocellular carcinomas (67% incidence) and papillary transitional cell carcinomas of the urinary bladder (83%) were observed in female dogs fed approximately 10.4 mg/kg/day orally in gelatin capsules over a period of 6.6-7.1 years (Stula et al. 1978). These tumors were absent in untreated controls. Although a small number of dogs (6) were evaluated, and only one sex and one dose were used, the significant increase in tumor rate in this group of dogs demonstrates unequivocally the carcinogenicity of this chemical in this species.

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

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

A synergistic role for 3,3'-dichlorobenzidine in the development of bladder cancer has been suggested. This was proposed in a study in which no carcinomas were found in any rats administered one of the following: 0.03% 3,3'-dichlorobenzidine in the diet, 0.001% BBN (N-butyl-N-(hydroxybutyl)nitrosamine) in drinking water, 0.0005% 2-acetylaminofluorene (2-AAF) in the diet, or 0.04% N-[4-(5nitro-2-furyl)-2-thiazolyllformamide (FANFT) in the diet for a period of 40 weeks (Ito et al. 1983). However, when BBN plus 3,3'-dichlorobenzidine were fed together at the same dose levels as above, there was a marked increase in the presence of papillary or nodular hyperplasia in the rat bladder, and the appearance of one papilloma. Based on these findings, the authors suggested that 3,3'-dichlorobenzidine had a synergistic effect on the carcinogenicity of BBN. In rats sequentially administered BBN (0.0l%), FANFT (0.15%), 2-AAF (0.025%), and 3,3'-dichlorobenzidine (0.03%) for 4 weeks each, the incidence of bladder cancer after administration of the 4 chemicals was no different than after administration of the first 3, suggesting no interactive effect of any type for 3,3'-dichlorobenzidine (Ito et al. 1983).

The Cancer Effect Level (CEL), (i.e., lowest dose that produced a tumorigenic response for each species) and the duration category of exposure to 3,3'-dichlorobenzidine are shown in Table 2-l and plotted in Figure 2-l. Based on the increased incidence in mammary adenocarcinomas in rats reported in the Stula et al. (1975) study, EPA calculated a q_1* of 0.45 $(mg/kg/day)^{-1}$. Doses corresponding to risk levels ranging from 10^{-4} to 10^{-7} are $2.2x10^{-4}$ to $2.2x10^{-7}$ mg/kg/day, respectively, as indicated in Figure 2-l.

2.2.3 Dermal Exposure

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

2.2.3.1 Death

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

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

2.2.3.2 Systemic Effects

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

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

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

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

Dermal Effects. Dermatitis was cited as the only verified health problem encountered by workers in contact with the free base of 3,3'-dichlorobenzidine in a dichlorobenzidine manufacturing plant (Gerarde and Gerarde 1974).

There was no discernable skin irritation when 3,3'-dichlorobenzidine dihydrochloride (at an unstipulated dose) was applied to the intact and abraded skin of rabbits (Gerarde and Gerarde 1974). Similarly, an

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

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

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

aqueous suspension of 3,3'-dichlorobenzidine instilled intradermally into rats at a dose of 700 mg/kg did not produce adverse effects (Gerarde and Gerarde 1974).

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

No effects were reported in rabbits when 100 mg of dichlorobenzidine (free base) was placed in the conjunctival sac of the eye (Gerarde and Gerarde 1974). It should be noted that the authors did not report the duration of exposure or the vehicle used. However, 0.1 mL of 3,3'-dichlorobenzidine dihydrochloride in a 20% corn oil suspension produced erythema, pus, and cornea1 opacity, giving a 76% score in the Draize test within an hour when placed in the conjunctival sac of the eye of the rabbit (Gerarde and Gerarde 1974). This response is very likely associated with the release of hydrochloric acid following the salt's contact with the moist surface of the eye.

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

2.2.3.3 Immunological and Lymphoreticular Effects

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

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

2. HEALTH EFFECTS

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

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

2. HEALTH EFFECTS

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

2.3 TOXICOKINETICS

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

2.3.1 Absorption

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

2.3.1.1 Inhalation Exposure

3,3'-Dichlorobenzidine has been detected in the urine of workers in 3,3'-dichlorobenzidine-handling plants under conditions which favored inhalation of 3,3'-dichlorobenzidine-bound particulate matter (Handke et al. 1986; London and Boiano 1986; Meigs et al. 1954). Under these conditions, it is reasonable to expect that some of the 3,3'-dichlorobenzidine found in the urine could have come from inhalation exposure. However, conditions in the plants were also conducive to dermal exposure. Therefore, some of the 3,3'-dichlorobenzidine dose found in the urine could have come from dermal exposure. In addition, since the mucocilliary clearance mechanism moves most of the larger particulates (5-10 μm) out of the lungs into

the gastrointestinal tract, it is reasonable to expect that some gastrointestinal dose was received as well. No information was located on absorption in animals following inhalation exposure.

2.3.1.2 Oral Exposure

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

In animals, absorption of 3,3'-dichlorobenzidine from the gastrointestinal tract is rapid. Following a dose of 40 mg/kg, the plasma level of unchanged 3,3'-dichlorobenzidine attained a peak concentration of 1.25 μg/mL at 4 hours in Sprague Dawley rats. Further, about 90% of the administered radioactivity was excreted in feces (via bile) and urine within 72 hours largely as metabolites, indicating a high bioavailability, typical of primary arylamines. The elimination is biphasic, with half-lives of 6 hours and 14 hours in plasma for the rapid and slow phases, respectively (Hsu and Sikka 1982).

2.3.1.3 Dermal Exposure

No studies were located regarding absorption of 3,3'-dichlorobenzidine following dermal exposure in humans. Because of large particle size and increased usage of closed systems and protective clothing, dermal absorption is minimized. In animals, dermally applied 3,3'-dichlorobenzidine (in acetone) is moderately absorbed. Based on the amount of radioactivity remaining at the site of application, the extent of dermal absorption of applied [^{14}C]-3,3'-dichlorobenzidine to the shaved skin of rats at 1, 8, and 24 hours following the application was estimated to be 6, 23, and 49%, respectively (Shah and Guthrie 1983).

2.3.2 Distribution

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

2.3.2.1 Inhalation Exposure

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

2.3.2.2 Oral Exposure

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

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

(Hsu and Sikka 1982). Overall, bioaccumulation of this chemical in rats is considered to be minimal following oral exposure of any duration.

There is indirect evidence that 3,3'-dichlorobenzidine or metabolites can cross the placenta. A study that examined the potential genotoxic effects of 3,3'-dichlorobenzidine found that oral administration of 3,3'-dichlorobenzidine to pregnant rats induced micronuclei in the liver of fetuses (Cihak and Vontorkova 1967). There is no information regarding accumulation of 3,3'-dichlorobenzidine or metabolites in breast milk or its potential transfer to offspring via breast milk.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution of 3,3'-dichlorobenzidine in humans following dermal exposure. The distribution of [^{14}C]-3,3'-dichlorobenzidine in rat tissues following dermal application was studied by Shah and Guthrie (1983). Tissues retaining >0.1% of the administered radioactivity 24 hours after application were liver (4.09%), blood (0.75%) and lung (0.45%). The level in the lung was the same at the 8- and 24-hour time points. Differences in the tissue distribution pattern of total radioactivity between the oral and dermal routes of 3,3'-dichlorobenzidine administration may be presumed to reflect differences in the rates of absorption from these sites. These differences suggest that the target organ in which 3,3'-dichlorobenzidine exerts an adverse effect may depend on the route of exposure to the compound. Organ toxicity can be better evaluated in comparative studies designed to test tissue distribution and persistence exposure.

2.3.3 Metabolism

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

Information from a study in which 4 volunteers ingested a single 250 mg dose of 3,3'-dichlorobenzidine suggests that this chemical undergoes *N*-acetylation and that metabolites may be excreted in the urine either free or as glucuronides (Belman et al. 1968). *N*-Acetylation appears to be the major path for the metabolism of 3,3'-dichlorobenzidine in mammals (Lazear et al. 1979; Reid et al. 1984; Tanaka 1981). Studies in animals also indicate that 3,3'-dichlorobenzidine is extensively metabolized. Bile and urine of rats given single oral doses of [^{14}C]-3,3'-dichlorobenzidine (40 mg/kg/day) contained 5 metabolites of 3,3'-dichlorobenzidine in addition to the parent compound. None of the metabolites were identified, but a majority were reported to be conjugates (Hsu and Sikka 1982). A 24-hour urine sample of rats given a

single oral dose of 3,3'-dichlorobenzidine (50 mg/kg/day) contained unchanged 3,3'-dichlorobenzidine, *N,N'*-diacetyl 3,3'-dichlorobenzidine, and *N*-acetyl 3,3'-dichlorobenzidine in a ratio of 1:3:10 (Tanaka 1981). Indirect evidence for the formation of nitroso derivatives was found in a study in which 3,3'-dichlorobenzidine was administered to female Wistar rats by gavage (Bimer et al. 1990). Hemoglobin adducts were detected by the release of 3,3'-dichlorobenzidine after alkaline hydrolysis. The authors stated that the most likely process by which the adducts were formed was a reaction between a nitroso derivative of 3,3'-dichlorobenzidine and sulfhydryls in cysteine residues of hemoglobin.

No studies were located regarding the metabolism of 3,3'-dichlorobenzidine in humans following dermal exposure. In a 24-hour urine sample of rats given a single dermal application of 3,3'-dichlorobenzidine (50 mg/kg/day), *N,N'*-diacetyl 3,3'-dichlorobenzidine (but not *N*-acetyl 3,3'-dichlorobenzidine or the unchanged chemical) was detected (Tanaka 1981). Since the utagenicity of diacetylated product is much less than either the monoacetylated or parent compound (Lazear et al. 1979; Reid et al. 1984; Tanaka 198l), diacetylation may be a detoxification reaction for 3,3'-dichlorobenzidine (see also Sections 2.4.1 and 2.4.2).

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

The metabolism of several 3,3'-dichlorobenzidine-based pigments has been studied in animal experiments to determine if they are metabolized to 3,3'-dichlorobenzidine. In a study where rats were exposed by inhalation to Pigment Yellow 17 (230 mg/m^3 air) for 4 hours, 3,3'-dichlorobenzidine was not detected in either urine or blood during the following 14 days (Hofmann and Schmidt 1993). No detectable residues of 3,3'-dichlorobenzidine were found in urine samples of hamsters administered a single dose of 100 mg/kg purified Yellow 12 (NCTR 1979; Nony et al. 1980). Similarly, 3,3'-dichlorobenzidine was not detected in

urine samples of rats fed 3,3'-dichlorobenzidine-derived pigments (C.I. Pigment Yellow 12, 16, and 83) in the diet at concentrations of 0.1% (1,000 ppm), 0.3% (3,000 ppm), and 0.9% (9,000 ppm) for 104 weeks (Leuschner 1978). Based on the results of these studies, there is no evidence for the metabolic cleavage of tested pigments to 3,3'-dichlorobenzidine in test animals (Hoffman and Schmidt 1993; Leuschner 1978; NCTR 1979; Nony et al. 1980).

2.3.4 Elimination and Excretion

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

2.3.4.1 Inhalation Exposure

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

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

2.3.4.2 Oral Exposure

Very limited information was located regarding excretion of 3,3'-dichlorobenzidine and/or metabolites in humans after oral exposure. In 4 volunteers who ingested a single 250 mg dose of 3,3'-dichlorobenzidine, the percentage of *N*-hydroxyacetyl compound excreted free in the urine in 24 hours ranged from 0.32 to 1.55%, whereas the percentage of *N*-hydroxyacetyl compound excreted as glucuronide in 24 hours ranged from 0.11 to 0.45% (Belman et al. 1968). Studies on the fate of 3,3'-dichlorobenzidinederived pigments fail to provide conclusive evidence that these pigments are broken down to release free 3,3'-dichlorobenzidine in humans.

Results from animal studies show that 3,3'-dichlorobenzidine administered by gavage is excreted primarily in feces and to a lesser extent in urine. In rats administered a single oral dose of [^{14}C]-3,3'-dichloro-

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

2.3.4.3 Dermal Exposure

No studies were located regarding the excretion of 3,3'-dichlorobenzidine in humans following dermal exposure. Fecal excretion in rats at 24 hours following 3,3'-dichlorobenzidine exposure was 19% of the administered dose, while urinary excretion accounted for 8% (Shah and Guthrie 1983). Fifty-one percent of the administered dose was unabsorbed from the site of application at 24 hours. The remaining 49% was distributed throughout the body, feces and urine.

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

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

2. HEALTH EFFECTS

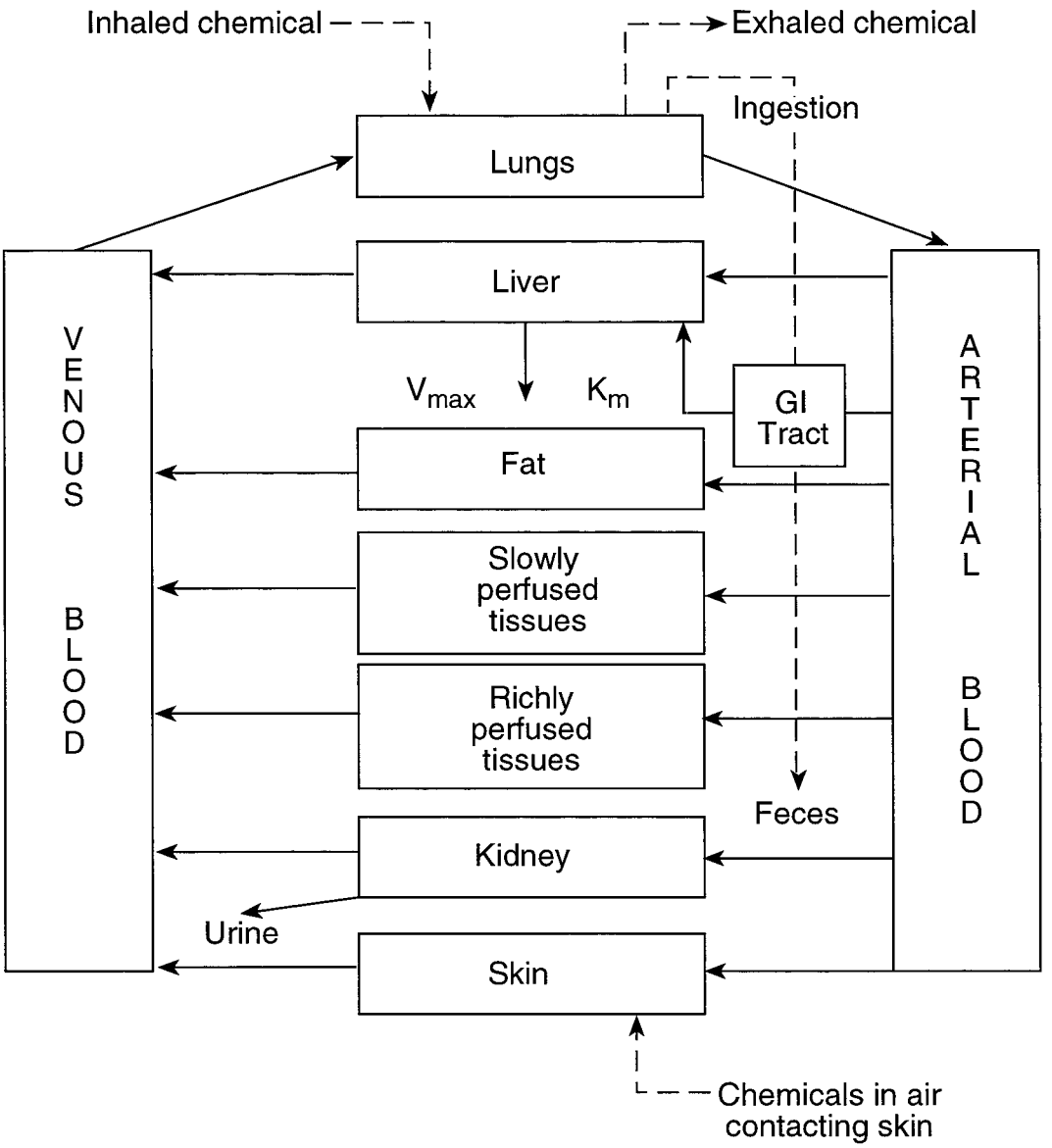
PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

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

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

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



Source: adapted from Krishnan et al. 1994

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

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

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

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

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

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

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

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

2.4.2 Mechanisms of Toxicity

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

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

2. HEALTH EFFECTS

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

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

There are data to suggest that 3,3'-dichlorobenzidine may act synergistically with other carcinogens. No carcinomas were found in any rats administered one of the following in the diet for a period of 40 weeks: 0.03% 3,3'-dichlorobenzidine in the diet, 0.001% BBN in drinking water, 0.0005% 2-AAF in the diet, or 0.04% FANFT (Ito et al. 1983). However, when BBN and 3,3'-dichlorobenzidine were fed together at the same dose levels as above, there was a marked increase in papillary or nodular hyperplasia in the rat bladder and the appearance of one papilloma. The authors suggested a synergistic effect of 3,3'-dichlorobenzidine on the carcinogenicity of BBN.

2.4.3 Animal-to-Human Extrapolations

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

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

2.5 RELEVANCE TO PUBLIC HEALTH

Overview.

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

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

Minimal Risk Levels for 3,3'-Dichlorobenzidine.

Inhalation MRLs.

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

Oral MRLs.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Dermal Effects. Dermatitis was cited as the only verified health problem encountered by workers in contact with the free base of 3,3'-dichlorobenzidine in a dichlorobenzidine manufacturing plant (Gerarde and Gerarde 1974). There was no discernable skin irritation when 3,3'-dichlorobenzidine dihydrochloride (at an unstipulated dose) was applied to the intact and abraded skin of rabbits (Gerarde and Gerarde 1974). Similarly, an aqueous suspension of 3,3'-dichlorobenzidine instilled intradermally into rats at a dose of 700 mg/kg did not produce adverse effects (Gerarde and Gerarde 1974). The observations in humans may have been allergic dermatitis, and specific protocols are required to make these determinations in laboratory animals.

Ocular Effects. No studies were located regarding ocular effects in humans after exposure to 3,3'-dichlorobenzidine by any route. No adverse effects on the eye were noted when dichlorobenzidine (isomer unspecified, free base) was directly placed in the conjunctival sac of the eye of rabbits (Gerarde and Gerarde 1974). However, 0.1 mL 3,3'-dichlorobenzidine dihydrochloride (dihydro salt of 3,3'-dichlorobenzidine) in a 20% corn oil suspension produced erythema, pus, and cornea1 opacity, giving a 76% score in the Draize test within an hour when placed in the conjunctival sac of the eye of the rabbit (Gerarde and Gerarde 1974). Apparently, the irritant effects of hydrochloric acid from the salt-compound contributed to the observed effects. Based on these data, it is not probable that adverse effects to the eye will occur in humans exposed to 3,3'-dichlorobenzidine at levels at which it occurs at hazardous waste sites.

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

2. HEALTH EFFECTS

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

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

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

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

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

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

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

In vivo, micronuclei were induced in polychromatic erythrocytes of the liver of fetal mice exposed transplacentally to the compound, and in liver cells of adult male mice treated orally with the compound at a maximum tolerated dose reported to be 1,000 mg/kg (Cihak and Vontorkova 1987). A sex difference in the genotoxicity of the compound is suggested, since adult male mice, but not pregnant females developed erythrocyte micronuclei following 3,3'-dichlorobenzidine exposure. However, whether this differential effect extends to carcinogenic effects is unclear. Positive chromatid exchange findings in an *in vitro* test system provide supportive evidence for 3,3'-dichlorobenzidine-induced cytogenetic changes. In a study using type I, II, and III Bloom Syndrome (BS) B-lymphoblastoid cell lines, 3,3'-dichlorobenzidine induced sister chromatid exchanges (SCEs) in all three types (Shiraishi 1986). However, the induction of SCE was variable among the three types. Exposure of BS type II and type III cells to 3,3'-dichlorobenzidine ($1x10^{-8}$ to $1.3x10^{-3}$ M) caused an increase in SCEs (120-140/cell) over baseline levels (70/cell) at the highest concentration ($1.3x10^{-3}$ M). BS type II cells required metabolic activation, while BS type III cells were sensitive with and without activation. The frequency of SCEs in BS type I cells was lower than in II and III.

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

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

Species (test system)	End point	Results	Reference
Mammalian cells			
Mouse bone marrow (male)	Micronuclei	+	Cihak and Vontorkova 1987
Mouse bone marrow (female)	Micronuclei	–	Cihak and Vontorkova 1987
Mouse fetal liver	Micronuclei	+	Cihak and Vontorkova 1987
Rat liver cells (male)	Unscheduled DNA synthesis	+	Ashby and Mohammed 1988
DNA Binding			
Mouse (male)	Binding to DNA	+	Ghosal and Iba 1990
Rat (male)	Binding to DNA	+	Ghosal and Iba 1990
Rat (male)	Binding to DNA	+	Bratcher and Sikka 1982

+ = Positive result; – = Negative result

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

Species (test system)	End point	Activation system	Result: With activation	Result: Without activation	Reference
Prokaryotic organisms:					
Salmonella typhimurium TA98	Gene mutation	Mouse liver S-9	+	+	Lazear et al. 1979
S. typhimurium TA98	Gene mutation	Hamster liver S-9	+	+	Savard and Josephy 1986
S. typhimurium TA98	Gene mutation	Rat liver S-9	+	ND	Vithayathil et al. 1983
S. typhimurium TA100	Gene mutation	Mouse liver S-9	–	–	Lazear et al. 1979
S. typhimurium NM2009	DNA damage	Mouse kidney S-9	+	ND	Imaoka et al. 1997
S. typhimurium NM2009	DNA damage	Mouse bladder S-9	+	ND	Imaoka et al. 1997
S. typhimurium NM2009	DNA damage	Mouse kidney CYP4B1	+	ND	Imaoka et al. 1997
S. typhimurium NM2009	DNA damage	Rat liver CYP4B1	+	ND	Imaoka et al. 1997
Eukaryotic organisms					
B-lymphoblastoid cell line II	Sister chromatid exchange	Rat liver S-9	+	–	Shiraishi 1986
B-lymphoblastoid cell line III	Sister chromatid exchange	Rat liver S-9	+	+	Shiraishi 1986

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

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

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

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

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

3,3'-Dichlorobenzidine formed adducts with calf thymus DNA when incubated with rat liver S9 (Bratcher and Sikka 1982), or horseradish peroxidase (Tsuruta et al. 1985) *in vitro*. 3,3'-Dichlorobenzidine was also shown to bind extensively to tissue DNA in rats and mice. Single oral administration of 20 or 100 mg/kg radiolabeled 3,3'-dichlorobenzidine to male Sprague-Dawley rats or Swiss-Webster mice resulted in extensive binding of the compound to tissue (liver, bladder, and intestine) DNA 12, 24, or 96 hours, and 9 or 14 days after treatment (Ghosal and Iba 1990). Results from *in vitro* studies in rats and mice indicated that 3,3'-dichlorobenzidine formed tissue DNA-binding derivatives of 3,3'-dichlorobenzidine (Ghosal and Iba 1990). However, the relevance of DNA adduct formation to the genotoxicity and carcinogenicity of the compound and to human health is not yet established. Therefore, the genotoxicity consequences of 3,3'-dichlorobenzidine in humans remain uncertain.

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

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

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

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

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

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

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

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

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

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

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

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

2.6 CHILDREN'S SUSCEPTIBILITY

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

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

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

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

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

No studies were located that specifically addressed the health effects of exposure to 3,3'-dichlorobenzidine in children. Limited data in adults are mostly derived from occupational studies with limitations including lack of precise exposure data and presence of other compounds. As a result, no organ or system has been identified as a target for 3,3'-dichlorobenzidine in humans, although dermatitis caused by skin contact with the free base was reported in one study (Gerarde and Gerarde 1974). It is reasonable to assume that the same effect would be seen in children similarly exposed. Because of the structural similarity of 3,3'-dichlorobenzidine with the known human bladder carcinogen benzidine, special attention has been paid to the incidence of bladder cancer among subjects occupationally exposed to 3,3'-dichlorobenzidine. Thus far, largely because of study limitations, there is no conclusive evidence that exposure to 3,3'-dichlorobenzidine increases the risk of bladder cancer in humans (Gadian 1975; Gerarde and Gerarde 1974; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996).

2. HEALTH EFFECTS

No studies were available that provided information on possible adverse developmental effects in humans exposed to 3,3'-dichlorobenzidine. The few available studies in animals were inadequate since they used parenteral administration of high doses of 3,3'-dichlorobenzidine (Golub 1970; Golub et al. 1975; Shabad et al. 1972).

There is no information regarding pharmacokinetics of 3,3'-dichlorobenzidine in children nor it is known whether 3,3'-dichlorobenzidine can be stored and excreted in breast milk. Although there have been no direct measurements to determine whether 3,3'-dichlorobenzidine can cross the placenta, there is some indirect evidence that it or its metabolites do. The evidence is based on the results of a study in which oral administration of 3,3'-dichlorobenzidine to pregnant mice resulted in the induction of micronuclei in the liver of fetuses (Cihak and Vontorvoka 1987). The results of another study in which subcutaneous administration of 3,3'-dichlorobenzidine to pregnant mice induced abnormal growth of the kidneys explanted from the fetuses also suggest that 3,3'-dichlorobenzidine or a metabolite can cross the placenta (Shabad et al. 1972). There is no information on whether 3,3'-dichlorobenzidine can be stored in maternal tissues and be mobilized during pregnancy or lactation, or whether it can reach parental germ cells.

There is no information on the metabolism of 3,3'-dichlorobenzidine in children. Limited data in humans suggest that N-acetylation is an important metabolic pathway (Belman et al. 1968), and a detoxification mechanism. N-Acetylation in humans is likely done by one of two families of N-acetyltransferases. One of these families, NAT2, is developmentally regulated (Leeder and Kearns 1997). Some enzyme activity can be detected in the fetus by the end of the first trimester. Almost all infants exhibit the slow acetylator phenotype between birth and 2 months of age. The adult phenotype distribution is reached by the age of 4-6 months, whereas adult activity is found by approximately l-3 years of age. Also, UDP-glucuronosyltransferase, responsible for the formation of glucuronide conjugates, seems to achieve adult activity by 618 months of age (Leeder and Keams 1997). These data suggest that metabolism of 3,3'-dichlorobenzidine by infants will differ from that in adults in extent, rate, or both.

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

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

2.7 BIOMARKERS OF EXPOSURE AND EFFECT

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

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

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate

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

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.9, Populations That Are Unusually Susceptible.

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

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

Hemoglobin adducts have been detected in female Wistar rats orally administered single 127 or 253 mg/kg doses of 3,3'-dichlorobenzidine (Birner et al. 1990). The investigators suggested that metabolically formed nitroso derivatives can result in the formation of a sulfinic acid amide with cysteine residues in hemoglobin (Birner et al. 1990). Hydrolysis yielded mainly 3,3'-dichlorobenzidine; N-acetylated 3,3'-dichlorobenzidine was also detected. Using a more sensitive analytical method, Joppich-Kuhn et al. (1997) also detected 3,3'-dichlorobenzidine-hemoglobin adducts in rats treated repeatedly with much lower doses (0.3-5.8 mg/kg/day) of 3,3'-dichlorobenzidine in the drinking water. The limit of detection of the method was below 0.1 ng/g hemoglobin and was linear up to 150 ng/g hemoglobin. Although these methods have not yet been validated in an occupationally exposed population, they appear potentially suitable for use as a biomarker of human exposure to 3,3'-dichlorobenzidine.

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

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

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

2.8 INTERACTIONS WITH OTHER CHEMICALS

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

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

FANFT (0.15%) 2-AAF (0.025%), and 3,3'-dichlorobenzidine (0.03%) for 4 weeks, the incidence ofbladder cancer after administration of the four chemicals was no different than after administration of the first three, suggesting no additive or antagonistic effect for 3,3'-dichlorobenzidine (Ito et al. 1983).

2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

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

2.10 METHODS FOR REDUCING TOXIC EFFECTS

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

2.10.1 Reducing Peak Absorption Following Exposure

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

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

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

2.10.2 Reducing Body Burden

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

2.10.3 Interfering with the Mechanism of Action for Toxic Effects

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

2.11 ADEQUACY OF THE DATABASE

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

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

all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

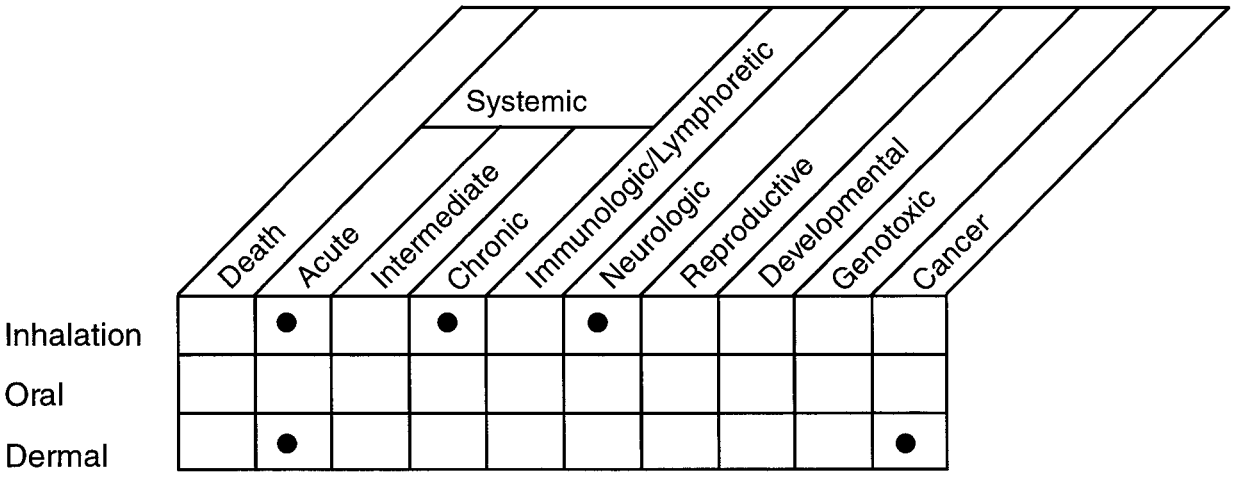
2.11 .l Existing Information on Health Effects of 3,3'-Dichlorobenzidine

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 3,3'-dichlorobenzidine are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of 3,3'-dichlorobenzidine. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Zdentifiing Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

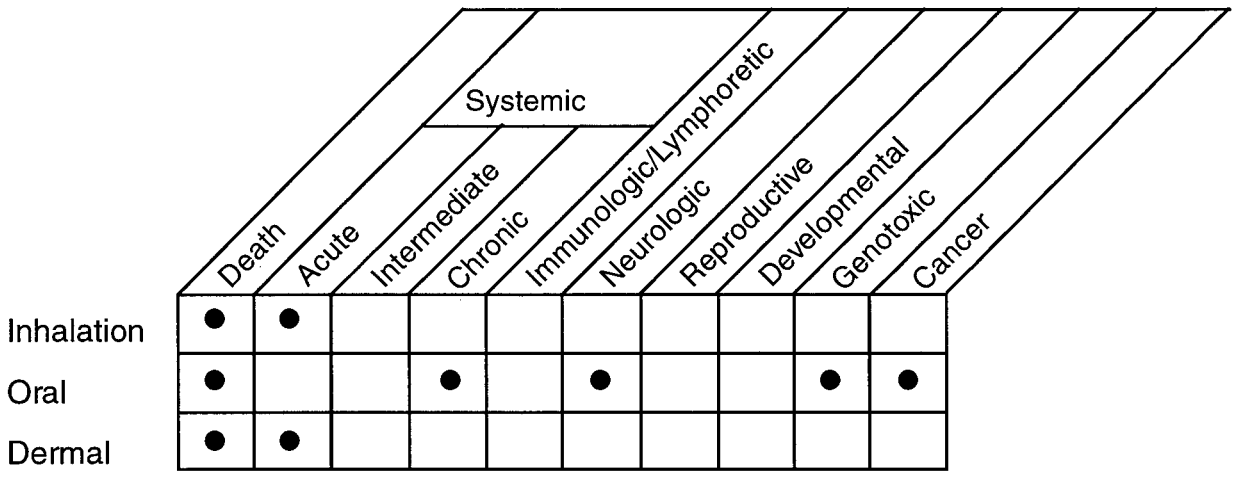
Essentially no studies of human exposure to 3,3'-dichlorobenzidine were located by specific routes, except for occupational data on direct dermal effects following dermal exposure and a recent carcinogenicity study in which skin contact with 3,3'-dichlorobenzidine and other arylamines was found to be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

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

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



Human



Animal

● Existing Studies

2.11.2 Identification of Data Needs

Acute-Duration Exposure. One study in humans showed that the compound may cause respiratory effects when inhaled and that application of 3,3'-dichlorobenzidine base causes skin irritation (Gerarde and Gerarde 1974). Thus, this limited information in humans is insufficient to conclusively identify target organs, other than the skin, following exposure by any route. Acute-duration exposure can cause eye damage (erythema, pus, cornea1 opacity) in rabbits following conjunctival application. However, the relevance of these findings for the general population is unknown since conjunctival application is not a typical route of exposure, and exposure by the inhalation route is unlikely. 3,3'-Dichlorobenzidine can be lethal following oral and dermal exposure at very high doses. In most animal studies, comprehensive gross and histopathological evaluations have not been conducted and clinical signs have not been monitored. Such studies may provide insight into systemic toxicity and potential health threat associated with acute-duration exposure. With the exception of effects caused by direct contact of 3,3'-dichlorobenzidine with the skin or the eyes, the limited pharmacokinetic data do not suggest route-specific target organs. The available data were inadequate for derivation of either inhalation or oral acute MRLs.

Intermediate-Duration Exposure. No intermediate-duration studies in humans were located. Intermediate-duration oral studies have been performed in rats without adverse systemic effects, but these studies used only one dose level (Griswold et al. 1968; Ito et al. 1983; Osanai 1976; Pliss 1959, 1963). Organs and/or tissues from the reproductive, neurological, and immunological systems have not been examined in the available intermediate-duration studies; such information would be useful. No intermediate-duration inhalation or dermal studies were found. Animal studies evaluating toxicological parameters at several dose levels would provide dose-response data which could prove more predictive when assessing potential adverse effects in humans following intermediate-duration exposure. No oral intermediate MRL was derived because the available studies did not identify relevant noncancer effects.

Chronic-Duration Exposure and Cancer. No studies were located that examined noncancer end points in humans following chronic exposure to 3,3'-dichlorobenzidine. Available chronic-duration oral studies provide information regarding systemic and carcinogenic effects in rats and dogs (Stula et al. 1975, 1978). These studies employed one dose level and toxicological parameters measured were limited. The inadequacies of these studies precluded derivation of a chronic oral MRL.
No chronic-duration animal inhalation or dermal exposure studies were located. Well conducted chronic-duration inhalation, dermal,

and oral studies involving low-dose exposure in animals might provide dose-response data on potential systemic effects of exposure in humans. The available data are insufficient to establish a relationship between the concentration of 3,3'-dichlorobenzidine and/or its metabolites in the body and the levels that are associated with adverse effects. Studies that provide data on the body burden of 3,3'-dichlorobenzidine associated with toxicity may prove useful.

Various studies have assessed the potential carcinogenicity of 3,3'-dichlorobenzidine in workers exposed to it (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996). However, many confounders have rendered the results inconclusive. A major difficulty in such studies is the simultaneous exposure to several potential or known carcinogens. The carcinogenicity of 3,3'-dichlorobenzidine has been well established in animals after oral administration of the compound (Osanai 1976; Pliss 1959, 1963; Sellakumar et al. 1969; Stula et al. 1975, 1978), but no information is available regarding inhalation and dermal exposure. There is suggestive evidence that 3,3'-dichlorobenzidine may cause cancer in animals when applied dermally since tumors were found in rats injected with the compound subcutaneously (Pliss 1963). Of particular interest would be additional studies, using relevant routes of exposure, to confirm the findings that 3,3'-dichlorobenzidine causes cancer in offspring of rats injected with the chemical subcutaneously during pregnancy (Golub et al. 1975)

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

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

Developmental Toxicity. No studies were found regarding developmental toxicity of 3,3'-dichlorobenzidine in humans. Animal studies have shown that 3,3'-dichlorobenzidine and/or metabolites may be transferred across the placenta and or through maternal milk to the offspring and may affect the growth of the kidneys after parenteral exposure during pregnancy (Golub 1972; Shabad et al. 1972) or induce tumors

in the offspring (Golub et al. 1975). The effects of the compound on development following oral, inhalation, or dermal exposure have not been studied. Well conducted animal studies employing various dose levels and relevant exposure routes during critical developmental periods may provide information on potential fetotoxicity, embryotoxicity, and teratogenic effects in humans. Also, cross-fostering studies may help determine the relative impacts of *in utero* transfer of the chemical and transfer through nursing. Further animal data may provide dose-response information if studies are conducted to determine what dose of 3,3'-dichlorobenzidine, or its metabolites, reaches the fetus.

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

Neurotoxicity. Based on its chemical structure, 3,3'-dichlorobenzidine does not appear to be neurotoxicant, but the nervous system has not been carefully evaluated after exposure to this chemical. Workers exposed to 3,3'-dichlorobenzidine (and to other chemicals as well) complained of headache and dizziness (Gerarde and Gerarde 1974). A chronic-duration oral study in dogs reported convulsions in one of six dogs treated orally with 3,3'-dichlorobenzidine (Stula et al. 1978). Upon necropsy, the authors noticed slight neuronal degeneration in tissues (unspecified) of the nervous system from this dog. However, the effect was seen in only one of the six dogs and only one dose level was tested. The limited information available does not suggest that 3,3'-dichlorobenzidine is a neurotoxicant, and studies aimed exclusively to evaluate this end point seem unnecessary at this time. However, any future long-term toxicity study on 3,3'-dichlorobenzidine in animals should include histological evaluation of representative elements of the nervous system. Furthermore, evaluation of neurological end points in offspring from animals exposed during gestation would provide information that may be relevant to children of pregnant women exposed to 3,3'-dichlorobenzidine in the workplace.

Epidemiological and Human Dosimetry Studies. The potential for occupational exposure exists in the use of 3,3'-dichlorobenzidine in the synthesis of 3,3'-dichlorobenzidine-based pigments for printing ink applications and to a lesser extent in paints. Workers exposed to 3,3'-dichlorobenzidine (and simultaneously to other chemicals) have complained of gastrointestinal upset, upper respiratory infection,

sore throat, caustic burns, headache, dizziness, and dermatitis (Gerarde and Gerarde 1974). The only one of these effects that appeared to be associated with 3,3'-dichlorobenzidine exposure with reasonable certainty is dermatitis, which was attributed to a manufacturing process change that resulted in exposure to dichlorobenzidine-freebase (Gerarde and Gerarde 1974). Studies of occupationally exposed individuals are complicated by the fact that there is usually simultaneous exposure to other chemicals. Based on available data, the potential for nonindustrial exposure to the general population by air, soil, or water is expected to be negligible. Epidemiological studies of people who live in areas where 3,3'-dichlorobenzidine has beendetected in groundwater, near industries releasing 3,3'-dichlorobenzidine, or near hazardous waste sites could provide information on whether 3,3'-dichlorobenzidine exposure produces effects in humans. In the unlikely event that exposure of the general population (in the past or present) primarily to 3,3'-dichlorobenzidine is identified, individuals should be monitored for gastrointestinal, respiratory, dermal, and neurological effects (as reported earlier by Gerarde and Gerarde 1974).

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

Biomarkers of Exposure and Effect.

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

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

Effect. There are no specific disease states in humans or animals that have been associated with exposure to 3,3'-dichlorobenzidine. Hemoglobin adducts have been isolated from the blood of 3,3'-dichlorobenzidine-treated animals (Birner et al. 1990; Joppich-Kuhn et al. 1997). It is not known what relationship exists between adduct levels in the blood and 3,3'-dichlorobenzidine toxicity. Further research in animal models is needed to determine if these adducts could be correlated with effects of 3,3'-dichlorobenzidine exposure. Further studies to identify more sensitive toxic effects (noncancer) that are specific for 3,3'-dichlorobenzidine would be useful in monitoring effects in people living near hazardous waste sites containing 3,3'-dichlorobenzidine.

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

Comparative Toxicokinetics. Pharmacokinetics studies have not been performed under conditions analogous to those of the carcinogenicity studies. Therefore, it is not possible to determine systemic levels of the compound associated with the reported effects. Pharmacokinetics data developed under exposure conditions associated with biological effects would markedly increase the possibility of improved species extrapolation for evaluating the true potency of 3,3'-dichlorobenzidine.

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

2. HEALTH EFFECTS

Children's Susceptibility. The information on health effects of 3,3'-dichlorobenzidine in humans is derived exclusively from studies of occupational exposure (Gadian 197.5; Gerarde and Gerarde 1974; MacIntyre 1975; Myslak et al. 1991; Quellet-Hellstron and Rench 1996). Because of study limitations such as simultaneous exposure to other chemicals, no target organ or system has been identified for 3,3'-dichlorobenzidine. In one occupational study it was reported that contact with the free base caused dermatitis (Gerarde and Gerarde 1974); it is reasonable to assume that children will respond in a similar manner under similar exposure conditions, although such exposure scenarios for children seem unrealistic. There is no information available to determine whether children and adults are equally susceptible to the toxic effects of 3,3'-dichlorobenzidine. No studies in animals have addressed this issue either, but given the unlikelihood of exposure to 3,3'-dichlorobenzidine by the general population, such studies do seem warranted at this time.

There is no information on whether the developmental process is altered in humans exposed to 3,3'-dichlorobenzidine. Studies in animals have been inadequate (Golub 1970; Golub et al. 1975; Shabad et al. 1972) and further well conducted research would be helpful to clarify whether the developmental process can be affected in animals exposed to 3,3'-dichlorobenzidine by a relevant route of exposure. This also includes information on whether 3,3'-dichlorobenzidine (or metabolites) can cross the placenta and/or be transferred to offspring via breast milk. There are no data to evaluate whether harmacokinetics of 3,3'-dichlorobenzidine in children are different from adults. There are no PBPK models for 3,3'-dichlorobenzidine, but a need for such a model is not apparent at this time. There is no information to evaluate whether metabolism of 3,3'-dichlorobenzidine in children is different than in adults, but there are some theoretical reasons to suspect that it might be different.

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

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

2.11.3 Ongoing Studies

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

3. CHEMICAL AND PHYSICAL INFORMATION

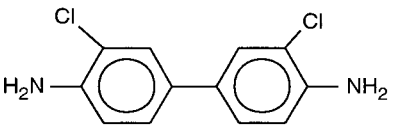
3.1 CHEMICAL IDENTITY

Information regarding the chemical identity of 3,3’-dichlorobenzidine is located in Table 3-l.

3.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of 3,3’-dichlorobenzidine is located in Table 3-2.

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

Characteristic	Information	Reference
Chemical name	3,3'-Dichlorobenzidine	Merck 1989
Synonym(s)	Dichlorobenzidine; 3,3'-dichloro(1,1'-biphenyl)-4,4'-diamine; 3,3'-dichloro-4,4'-biphenyldiamine	Merck 1989
Registered trade name(s)	Curithane	IARC 1982a
Chemical formula	$C_{12}H_{10}Cl_2N_2$	Merck 1989
Chemical structure		Merck 1989
Identification numbers:		
CAS Registry	91-94-1	Merck 1989
NIOSH RTECS	DD0525000	Chapman & Hall Database 1995
EPA Hazardous Waste	U073	HSDB 1996
OHM/TADS	8100004	HSDB 1996
DOT/UN/NA/IMCO	No data	
HSDB	1632	HSDB 1996
NCI	No data	

CAS = Chemical Abstracts Services; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances; IARC = International Agency for Research on Cancer

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

Property	Information	Reference
Molecular weight	253.13	Merck 1989
Color	Gray to purple	Merck 1989
Physical state	Crystalline solid	Lewis 1993
Melting point °C	132–133	Merck 1989
Boiling point °C	402	HSDB 1996
Density at 19 °C	No data	
Odor	No data	
Odor threshold: Air	No data	
Solubility: water at 20 °C organic solvent(s)	Almost insoluble 3.1 mg/L 4 mg/L (22 °C) Soluble in alcohol, benzene, Glacial acetic acid	Merck 1989 DCMA 1989 Banerjee et al. 1978 Merck 1989
Partition coefficients:		
Log K_{ow} Log K_{oc}	3.21 3.5 3.2 1.43–2.11 at pH7	SRC 1995b Nyman et al. 1997 Mabey et al. 1982 HSDB 1996
Vapor pressure at 20 °C	4.5 x 10^{-9} torr	DCMA 1989
Henry's law constant: at 25 °C	5.11 x 10^{-11} atm.m^3/mole	SRC 1994
Degradation half-life in air via reaction with OH radicals	9.7 hours = 39.5704x10^{-12} cm^3/molecule-sec	SRC 1995a
Dissociation constants $pK_{a,1}$ $pK_{a,2}$	 1.6 3.2	 Nyman et al. 1997 Nyman et al. 1997
Autoignition temperature, °C	No data	
Flashpoint	No data	
Flammability limits at 25 °C	No data	
Conversion factors (25 °C)	ppm = 0.0966 x mg/m^3 mg/m^3 = 10.35 x ppm	IARC 1982a
Explosive limits	No data	

4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

4.1 PRODUCTION

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

According to the 1997 Directory of Chemical Producers (SRI 1997), only one company, Lomac, Inc. of Muskegon, Michigan, manufactures (that is, produces) 3,3'-dichlorobenzidine. By contrast, in 1986, there were approximately 10 suppliers of the chemical listed in the United States (NTP 1994). Current production volumes of 3,3'-dichlorobenzidine for individual companies 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'-dichlorobenzidine-based dyes of over 18 million pounds in the United States. However, 3,3'-dichlorobenzidine is no longer used to manufacture dyes in the United States (CPMA 1998). Consumption of 3,3'-dichlorobenzidine in the United States amounted to 9.9 million pounds in 1987 (Hopmeier 1988).

Table 4-l lists the facilities in each state that manufacture 3,3'-dichlorobenzidine or process the compound for further distribution, the range of maximum amounts of 3,3'-dichlorobenzidine on-site, and the activities and uses of the product. "Processing" means the further distribution of the compound either as the same physical compound, in a different form or physical state, or as part of another article or mixture (40 CFR 372.3). In 1996, there was one facility in the United States that manufactured or used 3,3'-dichlorobenzidine. The data listed in Table 4-1 are derived from the 1996 Toxics Release Inventory (TR196 1998). Only certain types of facilities were required to report. Therefore, this is not an exhaustive list.

4.2 IMPORT/EXPORT

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

Table 4-1. Facilities That Manufacture or Process 3,3'-Dichlorobenzidine

FACILITY	LOCATION [a]	RANGE OF MAXIMUM AMOUNTS ON SITE IN POUNDS	ACTIVITIES AND USES
LOMAC INC.	MUSKEGON , MI	1,000 - 9,999	PRODUCE , IMPURITY

Source: TRI96 1998

[a] Post Office state abbreviations used

4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

4.3 USE

3,3'-Dichlorobenzidine 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 1979a). As of 1983, 7 specified pigments were commercially available. The yellow pigments derived from 3,3'-dichlorobenzidine can be used as substitutes for lead chromate pigments (HSDB 1996). Little, if any, dye is prepared from this compound. The chemical also has application as a compounding ingredient for rubber and plastics (HSDB 1996), and can be used to test for the presence of gold (Searle 1976). 3,3'-Dichlorobenzidine is used in the manufacture of the raw material tetraminobiphenyl which is used to produce polybenzimidazole (PBI). PBI fiber is used in many protective clothing applications, such as firefighter's apparel, welder's garments, high-temperature gloves, and crash rescue garments (Celanese 1985).

3,3'-Dichlorobenzidine is also used with 4,4'-methylenebis (2-chloroaniline) as a curing agent for liquidcastable polyurethane elastomers (HSDB 1996).

4.4 DISPOSAL

3,3'-Dichlorobenzidine 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 eyes. Nonetheless, some releases may occur in wastewater effluents.

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 the 3,3'-dichlorobenzidine to a quinone-type compound), and places them in polyethylene bags for disposal (London and Boiano 1986).

3,3'-Dichlorobenzidine is listed as a toxic substance under Section 313 of the Emergency Planning and Community Right to Know Act (EPCRA) under Title III of the Superfund Amendments and Reauthorization Act (SARA) (EPA 1995). Disposal of wastes containing 3,3'-dichlorobenzidine is controlled by a number of federal regulations (see Chapter 7). The current recommended technologies specified for treating 3,3'-dichlorobenzidine-containing wastes (waste waters and nonwastewaters) prior to land disposal include wet air oxidation, chemical or electrolytic oxidation, and carbon adsorption and incineration (EPA

1986). Facilities which generate 3,3'-dichlorobenzidine-containing wastes, and owners and operators of hazardous waste treatment, storage, and disposal facilities must also comply with regulations promulgated under the authority of the Resource Conservation and Recovery Act (RCRA).

5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

3,3'-Dichlorobenzidine is currently used in the production of insoluble dyes and pigments. Almost all 3,3'-dichlorobenzidine is now manufactured outside the United States and is imported for on-site processing or for use as a reactant to synthesize pigments. "Processing" means preparing a chemical after its manufacture for commercial distribution either as the same physical compound, in a different form or physical state, or as part of another article (for instance, a mixture) containing the chemical (40 CFR 372.3).

Use of the compound to synthesize soluble dyes ceased as of 1986, when better dyes from other sources were introduced. The distinction between dyes and pigments is not always clear. Pigments are almost without exception insoluble and exist as finely divided solid powders that are insoluble but wettable under the conditions of use. Dyes are almost always soluble organic substances used in coloring textiles or other fibrous substances.

Release routes of 3,3'-dichlorobenzidine to the environment appear to be waste waters, sludges, and solid wastes where emissions are not properly controlled during the use of 3,3'-dichlorobenzidine or during its chemical transformation to pigments. The compound has been found in water and soil at hazardous waste sites, a result of the improper land disposal of solid wastes.

Concern for human health derives primarily from inhalation of airborne dust or skin contact during careless handling or accidental spillage in occupational settings or drinking of contaminated well water by persons living in the proximity of hazardous waste sites. However, occupational case reports suggest that risk to workers exposed to 3,3'-dichlorobenzidine through the use of 3,3'-dichlorobenzidine-based pigments may be minimal. No adverse health effects were reported among 20 workers engaged in the manufacture and handling of 3,3'-dichlorobenzidine alone (concentration not specified) in a Japanese facility (DCMA 1989). No detectable levels of 3,3'-dichlorobenzidine or its monoacetyl metabolite (at a detection limit of 0.2 ppb) were seen in urine samples of workers who were exposed to pigments derived from 3,3'-dichlorobenzidine on the day the samples were collected (Hatfield et al. 1982). The urine analysis results for workers with

high potential for pigment exposure suggest that these pigments are not metabolized in humans although, without pigment exposure data, this conclusion is somewhat tentative.

The hydrochloric acid salt of 3,3'-dichlorobenzidine readily photolyses in water exposed to natural sunlight, but may not readily biodegrade in soil and acclimated sludges. It has a strong tendency to partition to soils and sediments, a property which reduces the potential for human exposure (Boyd et al. 1984; Chung and Boyd 1987; Sikka et al. 1978). Once partitioned to soil, the compound apparently binds further with humic substances to form humic-like materials that presumably would be non-hazardous (Sikka et al. 1978). However, in a recent paper, Nyman et al. (1997) stated that dehalogenation of 3,3'-dichlorobenzidine to form benzidine (also a toxic substance) occurs in sediment/water mixtures under anaerobic conditions. The compound does not volatilize or hydrolyze in solution, but it may slowly oxidize (Banerjee et al. 1978; Callahan et al. 1979). 3,3'-Dichlorobenzidine 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. 3,3'-Dichlorobenzidine accumulates in freshwater fish during aquatic exposure to either 5 ppb or 0.1 ppm concentrations of the chemical. After returning the fish to fresh, uncontaminated water, clearance of the compound from edible flesh was initially rapid (half-life of approximately 48 hours), but residues remained even after 14 days (Appleton and Sikka 1980). Steady-state concentrations in fish from ambient (unspiked) water exposures would be expected to be very low.

The reductive cleavage *in vivo* of azo dyes in general was first observed by Rinde and Troll (1975). Since then, several research groups have published articles that relate to the potential for human exposure to 3,3'-dichlorobenzidine that might arise via various chemical and biochemical mechanisms that degrade 3,3'-dichlorobenzidine-based synthetic dyes. A study by Hoffman and Schmidt (1993) found no evidence for metabolic cleavage of Pigment Yellow 17 to produced 3,3'-dichlorobenzidine in rats that inhaled the pigment. However, Zwirner-Baier and Neumann (1994), based on analysis of hemoglobin adducts from rats that drank the pigments, concluded that intestinal cleavage processes release very small amounts of 3,3'-dichlorobenzidine from Pigment Yellow 17 and Direct Red 46 (0.6% and 3%, respectively, of the total dose administered over 4 weeks). In another study (Sagelsdorff et al. 1996), the lack of appearance of 3,3'-dichlorobenzidine from Pigment Yellow 13 and 17 is shown, but a marked formation of 3,3'-dichlorobenzidine occurs from a soluble azo dye, C. I. Direct Red 46, which was an impurity in the pigments they studied.

5. POTENTIAL FOR HUMAN EXPOSURE

In metabolism studies of azo dyes and pigments in the hamster, in viva cleavage of the benzidine-based dye, Direct Black 38, to benzidine was shown by analysis of the urine. However, studies of the 3,3'-dichlorobenzidine-based pigment, Pigment Yellow 12, showed no evidence for *in vivo* cleavage to release 3,3'-dichlorobenzidine (Nony et al. 1980).

3,3'-Dichlorobenzidine has been identified in at least 32 of the 1,467 current or former EPA National Priorities List (NPL) hazardous wastes sites (HazDat 1998). However, the number of sites evaluated for 3,3'-dichlorobenzidine is not known. The frequency of these sites within the United States can be seen in Figure 5-l. The manufacture and use of 3,3'-dichlorobenzidine has been strictly regulated by OSHA since 1974. All work with the compound is done in closed systems and any residues are destroyed by chemical reaction. Such precautions, if conscientiously practiced, make it unlikely that significant quantities of 3,3'-dichlorobenzidine have been disposed of in landfills or at NPL sites after 1974.

NPL Superfund Records of Decision (RODS) were located for 24 of the 27 currently listed NPL sites where the HazDat database lists 3,3'-dichlorobenzidine as a contaminant. A ROD is a legally binding document that states the results of investigation and feasibility testing at hazardous waste sites and tells what techniques will be used to remediate the site. At four of the sites, 3,3'-dichlorobenzidine was verified as a contaminant. The RODS for the other 20 sites did not mention 3,3'-dichlorobenzidine as a contaminant of concern (i.e., one that warrants development of cleanup criteria and a choice of remedy). Affected soil was removed from three of the four contaminated sites. Only one site, Bofors Nobel in Michigan, required development of a cleanup criteria (CPMA 1998).

5.2 RELEASES TO THE ENVIRONMENT

According to the Toxics Release Inventory (TRI), in 1996, a total of 2 pounds (1 kg) of 3,3'-dichlorobenzidine was released to the environment from one processing facility (TR196 1998). Table 5-l lists amounts released from this facility. In addition, an estimated 250 pounds (118 kg) were released by manufacturing and processing facilities to publicly owned treatment works (POTWs), and an estimated 51,550 pounds (23,432 kg) were transferred offsite (TR196 1998). The TRI data should be used with caution because only certain types of facilities are required to report. Therefore, this is not an exhaustive list.

Table 5-1. Releases to the Environment from Facilities That Manufacture or Process 3,3'-Dichlorobenzidine

		Total of reported amounts released in pounds per year [a]						
STATE [b]	NUMBER OF FACILITIES	AIR [c]	WATER	LAND	UNDERGROUND INJECTION	POTW TRANSFER	OFF-SITE WASTE TRANSFER	TOTAL ENVIRONMENT [d]
MI	1	2	0	0	0	250	51,550	51,802

Source: TRI96 1998

[a] Data in TRI are maximum amounts released by each facility
[b] Post office state abbreviations used
[c] The sum of fugitive and stack releases are included in releases to air by a given facility
[d] The sum of all releases of the chemical to air, land, and water, and underground injection wells; and transfers off-site by a given facility

POTW = publicly-owned treatment works

5. POTENTIAL FOR HUMAN EXPOSURE

3,3'-Dichlorobenzidine has been identified in a variety of environmental media (air, surface water, groundwater, soil, and sediment) collected at 32 of the 1,467 current or former NPL hazardous waste sites (HazDat 1998). The frequency of these sites within the United States can be seen in Figure 5-l.

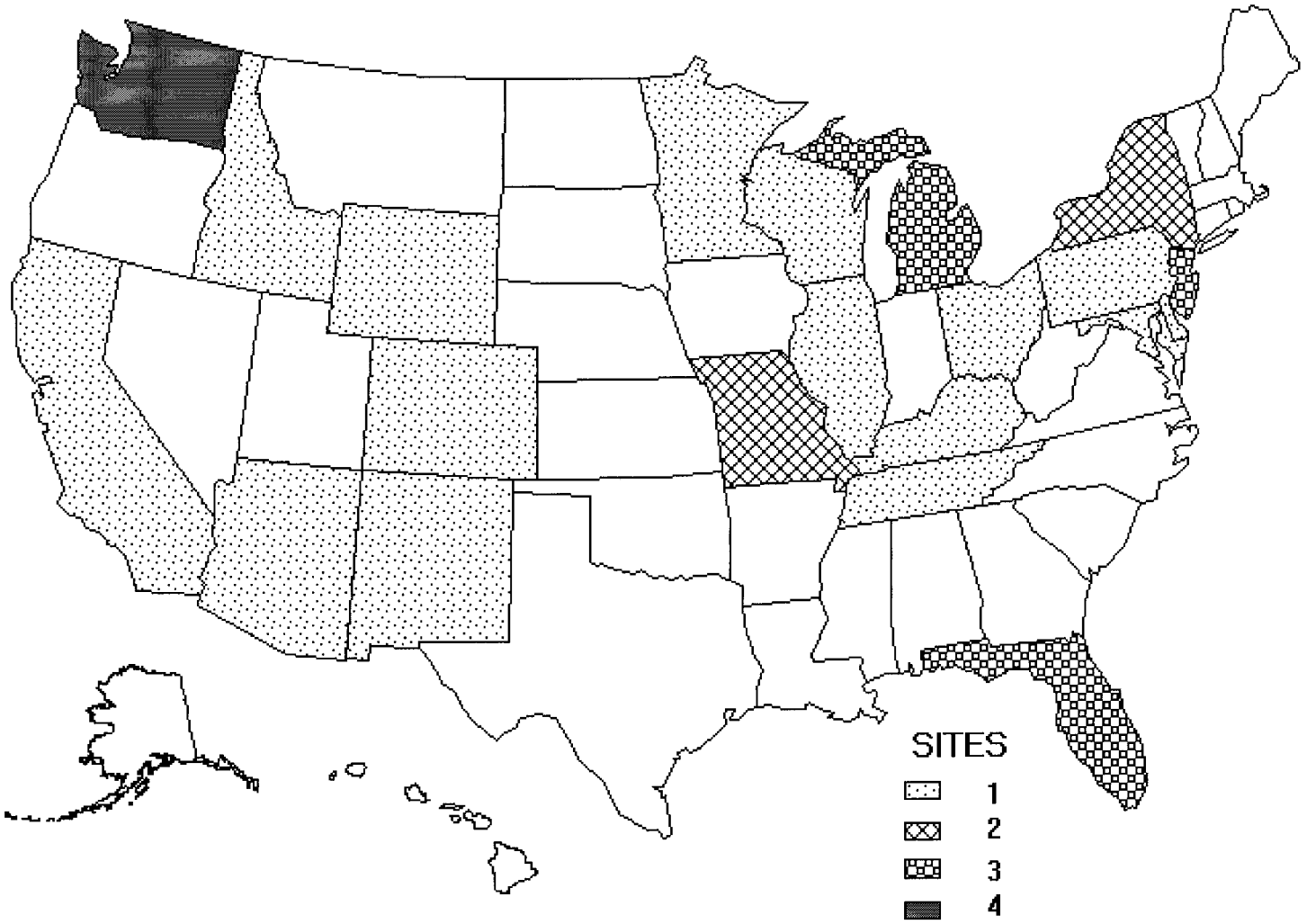
5.2.1 Air

The free base form of 3,3'-dichlorobenzidine is no longer utilized by industry in the United States. It is primarily supplied as the dihydrochloride salt (CPMA 1998). When it was used as the free base, it was handled as a powder or a moist paste (NIOSH 1980). 3,3'-Dichlorobenzidine is not a volatile chemical. A vapor pressure of $4.5x10x^{-9}$ mm Hg at 20 ºC has been reported (DCMA 1989). Prior to OSHA 1974 regulations, benzidine and 3,3'-dichlorobenzidine were manufactured in open systems that permitted atmospheric releases of suspended particles at the work site (Shriner et al. 1978), but no historical data were located specifically for 3,3'-dichlorobenzidine emissions (atmospheric or in water). The absence of data may be attributed to analytical methods used at that time 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 manufacturing systems are permitted, and atmospheric emissions are presumably reduced because of this regulation.

Estimated releases of 2 pounds (0.9 kg) of 3,3'-dichlorobenzidine to the atmosphere from one facility in 1996, accounted for 100% of the estimated total environment releases (TR196 1998). These releases are summarized in Table 5-1. The TRI data should be used with caution because only certain types of facilities are required to report information to the Toxics Release Inventory only if they employ more than 10 full-time employees, if their facility is classified under Standard Industrial Classification (SIC) codes 20 through 39, and if their facility produces, imports, or processes 25,000 or more pounds of any TRI chemical or otherwise used more than 10,000 pounds of a TRI chemical in a calendar year (EPA 1997). A member company of the Color Pigment Manufacturers Association, Inc., which monitors 3,3'-dichlorobenzidine under state regulations, reports that only *de minimus* values are found (CPMA 1998).

3,3'-Dichlorobenzidine was not identified in any air samples collected at any of the 32 NPL hazardous waste sites where it was detected in some other environmental media (HazDat 1998).

Figure 5-1. Frequency of NPL Sites with 3,3'-Dichlorobenzidine Contamination



Derived from HazDat 1998

5.2.2 Water

The free base form of 3,3'-dichlorobenzidine is sparingly soluble in water. The solubility of 3,3'-dichlorobenzidinem-2HCl in water is 4 mg/L at a pH of 6.9 (Banerjee et al. 1978). A solubility of 3.1 mg/L is also quoted (CPMA 1998). 3,3'-Dichlorobenzidine may be released into the environment in waste waters generated by the production of dyes and pigments.

No releases of 3,3'-dichlorobenzidine to the surface water were reported in 1996 (TR196 1998). Two hundred and fifty pounds (550 kilograms) were released to publicly owned treatment works (POTWs) (TR196 1998). These releases are summarized in Table 5-1. The TRI data should be used with caution because only certain types of facilities are required to report information to the Toxics Release Inventory only if they employ more than 10 full-time employees, if their facility is classified under Standard Industrial Classification (SIC) codes 20 through 39, and if their facility produces, imports, or processes 25,000 or more pounds of any TRI chemical or otherwise used more than 10,000 pounds of a TRI chemical in a calendar year (EPA 1997). As a result of secondary treatment processes in POTWs, only a small percentage of any 3,3'-dichlorobenzidine that might enter POTWs is subsequently released into surface water.

3,3'-Dichlorobenzidine has been identified in surface water and groundwater samples collected at 19 of the 32 NPL hazardous waste sites where it was detected in some other environmental media (HazDat 1998).

5.2.3 Soil

According to the Toxics Release Inventory, in 1996, there were no reported releases of 3,3'-dichlorobenzidine to soil from any large processing facilities (TR196 1998). The TRI data should be used with caution because only certain types of-facilities are required to report information to the Toxics Release Inventory only if they employ more than 10 full-time employees; if their facility is classified under Standard Industrial Classification (SIC) codes 20 through 39; and if their facility produces, imports, or processes 25,000 or more pounds of any TRI chemical or otherwise used more than 10,000 pounds of a TRI chemical in a calendar year (EPA 1997).

5. POTENTIAL FOR HUMAN EXPOSURE

3,3'-Dichlorobenzidine has been identified in soil and sediment samples collected at 18 of the 32 NPL hazardous waste sites where it was detected in some environmental media (HazDat 1998).

5.3 ENVIRONMENTAL FATE

Because 3,3'-dichlorobenzidine adsorbs to airborne dust particles or is otherwise bound to particulate matter, it is subject to dispersion, gravitational settling, and wash-out by rain. In water, 3,3'-dichlorobenzidine 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'-Dichlorobenzidine may be strongly adsorbed to 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). Although earlier research indicates that the compound does not appear to be readily biodegradable in soil or waste water sludges, recent work by Nyman (Nyman et al. 1997) indicates that more than 80% of 3,3'-dichlorobenzidine may be microbially degraded to benzidine under anaerobic conditions. 3,3'-Dichlorobenzidine is bioconcentrated by aquatic organisms under experimental conditions (Appleton and Sikka 1980), but it is not certain if it is bioaccumulated or transferred through the natural food chain.

5.3.1 Transport and Partitioning

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

The Henry's law constant for a compound is useful in estimating the partitioning of the compound between its vapor phase and aqueous media. At 25 ºC, a value of 5.11×10^{-11} atm-m^3/mole has been estimated (SRC 1994). This very low value suggests that 3,3'-dichlorobenzidine essentially remains dissolved in water, and does not migrate from water into air.

3,3'-Dichlorobenzidine 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. A K_{oc} value for 3,3'-dichlorobenzidine of 1,553 (based on an octanol-water partition coefficient (K_{ow}) of 3,236) was calculated by Mabey et al. (1982). This relatively high value implies that 3,3'-dichlorobenzidine would exhibit "low" mobility in soil (see Roy and Griffin 1985). However, 3,3'-dichlorobenzidine 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 an acid-base reaction, the amine groups may be protonated as follows:

$$3,3'\text{-DCB} + H_2O \leftrightarrow 3,3'\text{-DCBH}^+ + OH^-$$
$$3,3'\text{-DCBH'} + Hz0 \leftrightarrow 3,3'\text{-DCBH}_2^{2+} + OH^-$$

pK_a values reported for the conjugate acids (DCBH' and DCBH,") vary somewhat. Sikka (Sikka et al. 1978) and Boyd (Boyd et al. 1984) reported that they are <4. Nyman (Nyman et al. 1997) reported $pK_{a,1}$ and $pK_{a,2}$ values of 1.6 and 3.2, respectively. Thus, in the pH range of most environmental situations (pH 6-8) the dominant state of 3,3'-dichlorobenzidine in water would be the non-ionic form. As pH increases, the proportion of cationic forms of 3,3'-dichlorobenzidine decreases, and the extent of adsorption to sediments via Coulombic interactions would also decrease and 3,3'-dichlorobenzidine adsorption would be dominated by hydrophobic processes. This expectation was demonstrated by Sikka and coworkers (Sikka et al. 1978), who found that the adsorption constant (K_f) decreased with increasing pH; the decrease was more rapid in the range of pH 7-9. The adsorption data conformed to the Freundlich equation, $C_a = K_f C_s^{1/n}$ where C_a is the concentration of 3,3'-dichlorobenzidine adsorbed per mass of adsorbent, and C_s is the equilibrium concentration of 3,3'-dichlorobenzidine in solution. K_f and l/n are empirically derived constants. No correlation was found between K_f and the organic carbon content of the sediments (Boyd et al. 1984; Sikka et al. 1978). Similarly, 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). It was concluded that nonprotonated 3,3'-dichlorobenzidine is subject to hydrophobic bonding to some extent (Boyd et al. 1984). It is clear from these studies that adsorption constants for 3,3'-dichlorobenzidine cannot be accurately predicted for a given soil based only on a K_{oc} value.

The adsorption of 3,3'-dichlorobenzidine by soils and sediments may not be readily reversible (Boyd et al. 1984; Chung and Boyd 1987; Sikka et al. 1978). The extent of 3,3'-dichlorobenzidine desorption decreased with an increase in the age of the sample. Also, the adsorbed 3,3'-dichlorobenzidine was

resistant to extraction. After 24 hours of 3,3'-dichlorobenzidine-sediment contact, only 36% of the parent compound could be extracted by methanol. It is speculated that 3,3'-dichlorobenzidine forms covalent bonds with soil humic components (Sikka et al. 1978; Boyd et al. 1984). Experiments have indicated that covalent binding of ring-substituted anilines to humates is not a readily reversible reaction (Parris 1980). 3,3'-Dichlorobenzidine was highly immobile in soil column experiments (Chung and Boyd 1987). Water was passed through sandy soil (Entic Haplorthod) and 3,3'-dichlorobenzidine-contaminated sewage sludge samples. Only small amounts of radioactive 3,3'-dichlorobenzidine added to columns of sandy soil or sewage sludge were eluted with water over extended time periods. Extractable radioactivity from these soils and sludge samples decreased with time of chemical contact. There was greater adsorption of 3,3'-dichlorobenzidine to soil than to sludge, apparently as a result of the greater humus content of the soil samples, which suggested that the compound may favor migration from sludge to soil substrates (Chung and Boyd 1987).

Since 3,3'-dichlorobenzidine is lipophilic, it may be concentrated from aqueous media by aquatic organisms. Bluegill sunfish were exposed to radiolabeled 3,3'-dichlorobenzidine in dynamic-flow experiments for 130-168 hours (Appleton and Sikka 1980) . Moderately low bioconcentration factors (BCF) of 495-507 were calculated for the whole fish. BCFs in fish (golden ide) of 610 and in green algae of 940 have been reported (Freitag et al. 1985). A BCF in edible portions of bluegill sunfish of 114-170 has also been reported (EPA 1980b). Bioaccumulation by plants or terrestrial animals has not been studied. Assuming a log K_{ow} (range, 3.02-3.78) (DCMA 1989; Mabey et al. 1982) 3,3'-dichlorobenzidine is not likely to bioaccumulate appreciably. However, Law states that some bioaccumulation in aquatic organisms might be expected (Law 1995). The flesh of freshwater fish exposed to 5 ppb or 0.1 ppm concentrations of the chemical in water showed some accumulation. After returning the fish to clean water, clearance of the compound was rapid (a half-life of approximately 48 hours), but residues remained even after 14 days (Appleton and Sikka 1980).

5.3.2 Transformation and Degradation

5.3.2.1 Air

3,3'-Dichlorobenzidine in the sunlit, ambient air atmosphere may react with photochemically produced hydroxyl radicals and ozone, but there are no quantitative data on reaction rates. The persistence of "all

benzidines" in the atmosphere has been estimated by assuming a hydroxyl radical concentration of $8x10^{-10}$ mole/L (an average value in a 24-hour day-night cycle) (EPA 1975). Treating the oxidation process as a first-order reaction, the rate constant was $7.2x10^{12}$/mole-hour and the corresponding half-life was 12 hours. This estimation 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'-dichlorobenzidine in air has ranged from 1 to 60 days (EPA 1980b; Shriner et al. 1978). The most recently published value for the degradation half-life in air via reaction with OH radicals is 9.7 hours (SRC 1995a). The reason for this disparity among the half-life estimates is not known. No other information on the fate of 3,3'-dichlorobenzidine in the atmosphere was located.

5.3.2.2 Water

The limited information that is available suggests that 3,3'-dichlorobenzidine may photolyze in water to yield benzidine, which is more photostable yet still toxic. It does not appear that the chemical is susceptible to any other transformations in water except protonation by acid-base reactions.

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

It has been speculated that aqueous solutions of aromatic amines can be oxidized by organic radicals, but there are no actual data on reaction rates. Based on a study of reaction rate data for compounds with structures similar to 3,3'-dichlorobenzidine, an estimate of the half-life of aromatic amines in water is approximately 100 days, assuming a peroxy radical concentration of 10^{-10} mole/L in sunlit, oxygenated water (EPA 1975). Based on the oxidation rates of similar compounds, the direct oxidation of 3,3'-dichlorobenzidine by singlet oxygen in solution may be treated as a first-order reaction, to arrive at an estimated reaction constant of $<4x10^{7}$/mole-hour (Mabey et al. 1982). The oxidation rate constant with peroxy radicals was estimated to be approximately $4x10^{7}$/mole-hour. However, no information was located that demonstrates that 3,3'-dichlorobenzidine is significantly oxidized in water.

3,3'-Dichlorobenzidine was found to be extremely photolabile in water (Sikka et al. 1978; Banerjee et al. 1978). 3,3'-Dichlorobenzidine photolyzed yielding monochlorobenzidine, benzidine, and a number of

colored, water-insoluble products. In natural sunlight, the half-life of 3,3'-dichlorobenzidine in water was determined to be approximately 90 seconds. While 3,3'-dichlorobenzidine is very rapidly photolyzed under environmental conditions, the process may yield benzidine, a relatively photostable carcinogen (Banerjee et al. 1978).

3,3'-Dichlorobenzidine in lake water samples was not metabolized by microorganisms over a 4-week period (Sikka et al. 1978) although 1 lake sample of the 2 tested contained approximately 5 million microorganisms per mL. The composition of the biological community was not described. Minor decreases in 3,3'-dichlorobenzidine concentrations were attributed to adsorption onto suspended sediment.

5.3.2.3 Sediment and Soil

Earlier reports gave little indication that 3,3'-dichlorobenzidine is significantly degraded in soil or that it is transformed to other substances. More recent research (Nyman et al. 1997) reports that sediment/water mixtures spiked with 3,3'-dichlorobenzidine display evidence of the chemical's degradation. In the experiments reported by these authors, silty-clay to sandy sediments collected from a lake near Holland, Michigan, were spiked with 3,3'-dichlorobenzidine and incubated at 20 °C for 12 months under anaerobic conditions. Time-course analysis of this mixture showed that dehalogenation of 3,3'-dichlorobenzidine to produce benzidine appears to take place through a transient intermediate, 3-monochlorobenzidine. Up to 80% of the 3,3'-dichlorobenzidine was transformed to benzidine over a l-year incubation period. No metabolites were observed in autoclaved samples, suggesting that dehalogenation is mediated by microbial activity. The final product, benzidine, shows more affinity for the solution (aqueous) phase and thus has a greater potential for transport in the environment.

Unsubstituted benzidine may be oxidized at clay surfaces when mixed with some types of clay minerals (Tennakoon et al. 1974; Theng 197 1). 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'-dichlorobenzidine is subject to the same type of surface oxidation at solid-liquid interfaces.

5. POTENTIAL FOR HUMAN EXPOSURE

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

The results of seven laboratories conducting aerobic biodegradation experiments with 3,3'-dichlorobenzidine have been summarized (Brown and Laboureur 1983). 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. The authors hypothesize that the yeast may be a food source to allow buildup of large concentrations of active bacteria that are able to break down the amines. The authors felt that these results showed the "inherent biodegradability" of 3,3'-dichlorobenzidine, but that the compound should not be classified as "readily biodegradable" (Brown and Laboureur 1983). Possible degradation mechanisms and degradation by-products were not discussed.

3,3'-Dichlorobenzidine degraded very little when incubated with soil. In a study by Boyd et al. (1984), a Brookston clay loam soil (a typic Argiaquoll fine loamy, mixed mesic) containing [^{14}C]-3,3'-dichlorobenzidine at concentrations of 40 and 4 mg/kg of dry soil was incubated aerobically and anaerobically in batch experiments (Boyd et al. 1984). Under aerobic conditions, 3,3'-dichlorobenzidine 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 1 year of incubation. The authors did not comment on the population or type of microorganisms in the soil sample (Boyd et al. 1984). Additional studies indicated that 3,3'-dichlorobenzidine was very persistent in soil and sludge-amended soil (Chung and Boyd 1987). Biodegradation of [^{14}C]-3,3'-dichlorobenzidine 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'-dichlorobenzidine recovered as $^{14}CO_2$ was <2%. It should be noted that biodegradation when measured by $^{14}CO_2$ evolution may provide 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 for the compound being only partially metabolized (Graveel et al. 1986). The disparity between the results of this work and the results of Nyman (Nyman et al. 1997) is probably related to the nature of their respective biotic communities.

5. POTENTIAL FOR HUMAN EXPOSURE

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to 3,3'-dichlorobenzidine depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. In reviewing data on 3,3'-dichlorobenzidine levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for detection and measurement of 3,3'-dichlorobenzidine are detailed in Chapter 6.

3,3'-Dichlorobenzidine was not detected in the 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'-dichlorobenzidine 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 use of 3,3'-dichlorobenzidine-based dyes has decreased to zero over the last 30 years, while environmental and health regulations have been implemented to reduce the release of 3,3'-dichlorobenzidine to the environment.

5.4.1 Air

3,3'-Dichlorobenzidine does not naturally occur in the environment (IARC 1982a). 3,3'-Dichlorobenzidine 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). More recent data on occupational exposure levels indicate the presence of levels ≤0.6-2.5 μg/m^3 in 3,3'-dichlorobenzidine production and pigment manufacturing plants in Germany (DCMA 1989).

The concentration of 3,3'-dichlorobenzidine in the Canadian environment was estimated by Liteplo and Meek (1994) by applying the Level III Fugacity Computer Model of Mackay and Paterson (Mackay and Paterson 1991). Assuming that 1% of the total amount produced in and imported to Canada is released into various media in proportions similar to those given in the U.S. TRI, the average concentration of 3,3'-dichlorobenzidine in air, as estimated by the model, is $7.6x10^{-16}$ μg/m^3.

5.4.2 Water

EPA's computerized water quality database (STORET) was used to determine the median concentration of 3,3'-dichlorobenzidine in surface water, groundwater, and municipal and industrial inflow and outflow (Staples et al. 1985). The median concentration of 3,3'-dichlorobenzidine detected in 12 of 1,239 samples of waste effluent collected from about 1980 to 1984, was reported to be <10 ppb. The median concentration of 3,3'-dichlorobenzidine in both surface and groundwater was also reported to be <10 ppb. The EPA reported that water samples collected from drinking-water wells near a waste disposal lagoon that contained 3,3'-dichlorobenzidine-manufacturing wastes had concentrations of the chemical ranging from 0.13 to 0.27 ppm (EPA 1980b). EPA indicated that 3,3'-dichlorobenzidine concentrations in waste waters from metal finishing operations were 0.07 ppb or less (EPA 1983c). Discharge concentrations from other industrial sources were at most 10 ppb. Using a Fugacity Computer Model, Liteplo and Meek estimated the concentration of 3,3'-dichlorobenzidine in Canadian water to be $3.4x10^{-7}$ ng/L (Liteplo and Meek 1994). Because the model does not address the possibility of bound residue in sediment, the concentration in water is certainly overestimated.

Capillary gas chromatography/mass spectrometry (GC/MS) was used to identify, but not quantify, 3,3'-dichlorobenzidine in the dissolved phase (that is, smaller particles and dispersed colloids not retained by the filter) of water concentrates from the Besos River in Spain (Grifoll et al. 1992). Valls et al. identified 3,3'-dichlorobenzidine in urban wastewater in the same region (Valls et al. 1990).

5.4.3 Sediment and Soil

The estimated median concentration of 3,3'-dichlorobenzidine in sediments in the United States has been reported to be <l ppm on a dry sediment basis (Staples et al. 1985). Of the 34 sediment or soil measurements recorded in the STORET database, none of the samples contained detectable concentrations of 3,3'-dichlorobenzidine.

5.4.4 Other Environmental Media

There is a potential for 3,3'-dichlorobenzidine to occur in waste water sludges and industrial solid wastes. A 3,3'-dichlorobenzidine concentration of 16 ppm in municipal sludge from Michigan has been reported

(Chung and Boyd 1987). 3,3'-Dichlorobenzidine was detected at concentrations of 3.13 mg/kg dry sewage sludge in 2 of a total of 253 sewage treatment plants examined (Fricke et al. 1985). These plants were all in the United States (Arizona, Indiana, Michigan, Missouri, New Mexico, New York, and Texas). Concentrations up to 535 μg/L were detected in a 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 in Muskegon, Michigan (Demirjian et al. 1984).

Because the chemical has no agricultural or food chemical application, it is very unlikely that 3,3'-dichlorobenzidine occurs in food in general. [^{14}C]-3,3'-Dichlorobenzidine was found to rapidly accumulate in bluegill sunfish as a result of their exposure to water in which either 5 or 100 μg/L of the chemical was intentionally added. Residues were distributed in both the edible and nonedible portions (Appleton and Sikka 1980). However, 3,3'-dichlorobenzidine was not detected in fish samples obtained from rivers near nine textile dyestuff manufacturers known to use 3,3'-dichlorobenzidine-based pigments (Diachenko 1979).

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Years ago, benzidine and its congeners such as 3,3'-dichlorobenzidine were likely to be found only in the vicinity of pigment plants (EPA 1980b; Shriner et al. 1978) where wastes may escape or be discharged. 3,3'-Dichlorobenzidine may also be found in locations where it is used in formulating other products such as rubber and plastic (HSDB 1996) or in producing polybenzimidazole (PBI) (Celanese 1985). However, 3,3'-dichlorobenzidine is no longer used to manufacture soluble dyes in the United States (CPMA 1998). Based on available data, the potential for nonindustrial exposure via air, soil, or water is expected to be negligible. The greatest chance of exposure by the general public is from the improper land disposal of compounds. The significance of this exposure route can only be evaluated on a site-by-site basis.

No uses of 3,3'-dichlorobenzidine in commonplace consumer products are known. In the past, the general public may have been exposed to minute amounts of 3,3'-dichlorobenzidine during the use of pressurized spray containers of paints, lacquers, and enamels containing traces of benzidine yellow, a pigment derived from 3,3'-dichlorobenzidine (Shriner et al. 1978). 3,3'-Dichlorobenzidine-based pigments are normally used in printing ink applications; their use in paints is rare and, thus, its presence in present-day pressurized paint spray would not be expected (CPMA 1998).

5. POTENTIAL FOR HUMAN EXPOSURE

Today the most likely possibilities for occupational exposure exist in the processing of 3,3'-dichlorobenzidine in the synthesis of pigments, the compounding of PBI, and for workers in the garment, leather, printing, paper, and homecraft industries where benzidine-based pigments are used. However, there appears to be no information available on current levels of occupational exposure in the United States. Since 1974, OSHA regulations have set strict standards for worker protection, required the use of closed manufacturing vessels, and prescribed methods to chemically destroy residues. Although there is limited evidence for *in vivo* cleavage of 3,3'-dichlorobenzidine-derived pigments to free 3,3'-dichlorobenzidine in animals, urinary tract data from pigment workers suggest that 3,3'-dichlorobenzidine-derived pigments are not significantly metabolized in humans. Less than 0.2 ppb of 3,3'-dichlorobenzidine was detected in urine samples of 36 workers exposed to pigments derived from the compound (Hatfield et al. 1982).

In Canada, the estimated daily intake of 3,3'-dichlorobenzidine by various segments of the population has been calculated. The calculations are based on the predicted levels of 3,3'-dichlorobenzidine in air, water, and soil, as well as on the estimated daily intake of each (air, water, soil) by Canadians (Government of Canada 1993). The predicted concentrations or human intake levels are not measured values but rather predicted values based on output from mathematical models using worst-case assumptions that do not take into consideration removal mechanisms such as photolysis, oxidation, or irreversible binding to substrates. The total intake by adults (20 or more years of age) is predicted to be $7.4x10^{-9}$ ng/kg body weight/day. For infants up to 6 months of age (the group with the greatest predicted exposure on the basis of body weight), the total intake is estimated at $3.6x10^{-8}$ ng/kg body weight/day.

5.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans and briefly considers potential pre-conception exposure to germ cells. Differences from adults in susceptibility to hazardous substances are discussed in Section 2.6, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, and breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior

and lifestyle also influence exposure. Children crawl on the floor; they put things in their mouths; they may ingest inappropriate things such as dirt or paint chips; they spend more time outdoors. Children also are closer to the ground, and they do not have the judgment of adults in avoiding hazards (NRC 1993).

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

Children sometimes put dirt in their mouths. Because the adsorption of 3,3'-dichlorobenzidine to soils and sediments may not be readily reversible (Boyd et al. 1984; Chung and Boyd 1987; Sikka et al. 1978), the bioavailability of the compound is limited. A child who ingested contaminated dirt would be expected to incur less exposure as compared to that from other, more direct routes.

Another potential exposure route for children is through exposure to clothing and tracked-in dirt brought in by parents who work in factories that produce 3,3'-dichlorobenzidine. A public health assessment study conducted in Michigan in 1981 (ATSDR 1996) found the compound in the homes of 9 employees. Samples collected from vacuum cleaner bags had up to 10.5 ppm and dryer lint contained up to 0.74 ppm. If these homes have not been adequately cleaned, exposure could continue.

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

In addition to individuals who are occupationally exposed to 3,3'-dichlorobenzidine (see Section 5.5) there are several groups within the general population that have the potential for exposures to 3,3'-dichlorobenzidine at levels above those of the general population. These groups include individuals living in proximity to sites where 3,3'-dichlorobenzidine was produced or sites where 3,3'-dichlorobenzidine was disposed, and individuals living near one of the 32 NPL hazardous waste sites where 3,3'-dichlorobenzidine has been detected in some environmental media (HazDat 1998). 3,3'-Dichlorobenzidine was not detected in fish samples obtained from rivers near nine textile dyestuff manufacturers known to use 3,3'-dichloro-

benzidine-based pigments (Diachenko 1979), nor were there any fish consumption advisories for 3,3'-dichlorobenzidine in 1996. Therefore, recreational and subsistence fishers are not at risk.

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

5.8 ADEQUACY OF THE DATABASE

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

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

5.8.1 Identification of Data Needs

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

system under study. Because there is some discrepancy regarding the volatility of the free base form of 3,3'-dichlorobenzidine (Gerarde and Gerarde 1974; CPMA 1998) research in this area is indicated.

Production, Import/Export, Use, Release, and Disposal. According to the Toxics Release Inventory (TRI) report (TR196 1998), 3,3'-dichlorobenzidine is manufactured at one facility in Michigan. Three of the five facilities listed by TR196 that process the compound depend on imports for their supply. The chemical is no longer used to produce dyes in the United States (better dyes based on other chemicals are available); its main use is in the production of pigments (DCMA 1989). It also finds some use in the formulation of rubber and plastic (HSDB 1996) and in the production of PBI (Celanese 1985). The compound is not used in the home or in the open environment; however, there is evidence that the compound can be brought into the home on the shoes and clothing of adults who work with 3,3'-dichlorobenzidine (ATSDR 1996) but the quantity that might be present is unknown. In the workplace, OSHA regulations require that the compound be handled in closed systems and that shipping containers be cleaned thoroughly (again, within a closed system) before disposal (DCMA 1989). The free base or salt form of the compound is not used in the home or in the general environment. It is handled only by industry to make pigments; thus there seems to be little chance the chemical could contaminate the food supply. No evidence of the compound in fish taken downstream from nine facilities known to handle 3,3'-dichlorobenzidine was found (Diachenko 1979). Citations regarding disposal techniques for 3,3'-dichlorobenzidine are found in the Hazardous Substances Data Base (HSDB). Small quantities can be destroyed by chemical reaction, for example, with sodium hypochlorite solution, which converts 3,3'-dichlorobenzidine to a quinone-type compound. Incineration at high temperatures can be used to destroy work garments and miscellaneous solid wastes exposed to the compound. Presumably only small amounts would need to be disposed since the compound is mainly consumed by conversion to pigments.

According to the Emergency Planning and Community Right-to-Know Act of 1986,42 U.S.C. Section 11023, industries are required to submit chemical release and off-site transfer information to the EPA. The TRI, which contains this information for 1996, became available in May of 1998. This database will be updated yearly and should provide a list of industrial production facilities and emissions.

Environmental Fate. It is not known if 3,3'-dichlorobenzidine, like benzidine, is oxidized by clay minerals or if cations in water can have the same oxidizing effect. 3,3'-Dichlorobenzidine does not appear to biodegrade easily, 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. A recent study (Nyman et al. 1997) provides evidence that in the span of a year up to 80% of 3,3'-dichlorobenzidine can degrade to benzidine in anaerobic mixtures of sediment/water. Further research to identify the pathways and products of decomposition of 3,3'-dichlorobenzidine in various soils is needed. The toxicological profile for benzidine contains information on the environmental fate of that compound (ATSDR 1995).

Bioavailability from Environmental Media. No information on the presence of 3,3'-dichlorobenzidine in foods was located in the available literature. The Canadian Government's Priority Substances List Assessment Report for 3,3'-dichlorobenzidine (Government of Canada 1993) also reports that no data on the levels of 3,3'-dichlorobenzidine in drinking water or foodstuffs were identified within either Canada or the United States. Because 3,3'-dichlorobenzidine has been found to bind strongly to soil constituents (Berry and Boyd 1985; Chung and Boyd 1987), Law (1995) concluded that it would also bind strongly to sedimentary material in the marine aquatic environment and thus may have limited bioavailability.

Food Chain Bioaccumulation. 3,3'-Dichlorobenzidine is bioconcentrated by aquatic organisms under experimental conditions. Whole-fish BCFs of around 500, with equilibration occurring in 96-168 hours, have been published (Appleton and Sikka 1980). In view of the n-octanoywater partition coefficient for 3,3'-dichlorobenzidine, limited bioaccumulation could be expected (Law 1995) because the retention time of the chemical in exposed fish is short (Appleton and Sikka 1980). The ability of aquatic organisms to concentrate the compound could present a human health hazard if contaminated fish were eaten. However, 3,3'-dichlorobenzidine was not found in fish taken from waters in the vicinity of dye or textile manufacturing plants on the Buffalo and Delaware rivers in the United States (Diachenko 1979). It was concluded that monitoring for 3,3'-dichlorobenzidine in marine waters of the United Kingdom is unwarranted at present (Law 1995).

Exposure Levels in Environmental Media. There were no quantitative data on current atmospheric levels of 3,3'-dichlorobenzidine emissions or on the chemical's potential to act as a surface contaminant of soil environments. It is difficult to determine 3,3'-dichlorobenzidine 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 ascertain fully the environmental fate of 3,3'-dichlorobenzidine as analytical advances permit the routine determination of very low concentrations. Moreover, determination of the nature and environmental fate of breakdown products of 3,3'-dichlorobenzidine would be useful.

5. POTENTIAL FOR HUMAN EXPOSURE

Reliable monitoring data for the levels of 3,3'-dichlorobenzidine in contaminated media at hazardous waste sites are needed so that the information obtained on levels of 3,3'-dichlorobenzidine in the environment can be used in combination with the known body burdens of 3,3'-dichlorobenzidine to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. It has been speculated that the 1974 OSHA regulations have reduced workplace air levels of 3,3'-dichlorobenzidine (CPMA 1998). However, it would be important to conduct exposure studies to monitor air levels in the workplace to confirm this premise. The need for more information on the extent of air, water, and soil contamination by industrial plant emissions or waste sites containing 3,3'-dichlorobenzidine continues. There is little information on exposure of children to 3,3'-dichlorobenzidine (or products derived from the compound). The compound has a very limited distribution and is not present in consumer goods (other than in insoluble pigmented forms). This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. There is no available information on exposure of children to 3,3'-dichlorobenzidine (or products derived from the compound). The compound has a very limited distribution and is not present in consumer goods (other than in insoluble pigmented forms). Thus, there is no pressing need to gather data related to children's exposure. However, given sufficient resources, the topic of inadvertent take-home exposure by occupationally exposed parents could be explored. A public health assessment (ATSDR 1996) found measurable levels of 3,3'-dichlorobenzidine (10.5 ppm in vacuum cleaner bags and 0.74 ppm in clothes dryer lint) in the homes of workers who were employed in manufacturing or processing the compound.

Exposure Registries. No exposure registries for 3,3'-dichlorobenzidine were located. This substance is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for subregistries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

5.8.2 Ongoing Studies

No information was located regarding ongoing studies.

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring 3,3'-dichlorobenzidine, its metabolites, and other biomarkers of exposure and effect to 3,3'-dichlorobenzidine. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL SAMPLES

Methods for the determination of 3,3'-dichlorobenzidine and its metabolites in biological materials are summarized in Table 6- 1.

The compound 3,3'-dichlorobenzidine has been measured most often in urine and serum using gas chromatography (GC) (Bowman and Nony 1981; Bowman and Rushing 1981; Hoffman and Schmidt 1993; Joppich-Kuhn et al. 1997; Nony and Bowman 1980; Nony et al. 1980) and high performance liquid chromatography (HPLC) (Birner et al. 1990; Bowman and Nony 1981; CPMA 1998; Nony and Bowman 1980; Nony et al. 1980; Zwirner-Baier and Neumann 1994). A method for 3,3'-dichlorobenzidine in fish using GC (Diachenko 1979) has been reported. GC methods usually relied upon selective detection of the fluorinated derivatives while HPLC methods relied on absorbence or electrochemical detection. In addition, one method of analysis in urine used a spectrophotometric approach (Roberts and Rossano 1982). Several of the reported methods can also be used to determine the mono- and di-acetylated metabolites. The studies of Birner et al. (1990), Joppich-Kuhn et al. 1997, and Zwirner-Baier and Neumann (1994) reported the determination of 3,3'-dichlorobenzidine and monoacetyl-3,3'-dichlorobenzidine following hydrolysis of the analyte-hemoglobin adducts (the adduct is a marker of exposure). Although most of these methods have been developed using animal samples, they should also be applicable to the determination of 3,3'-dichlorobenzidine and its metabolites in samples of human origin. Limits of detection in the low to mid ppb range

Table 6-1. Analytical Methods for Determining 3,3'-Dichlorobenzidine and Metabolites in Biological Samples

Sample type	Extraction/cleanup	Detection	Limit of detection	Percent recovery	Reference
Human hemoglobin adducts (dichloro-benzidine, monoacetyl-dichlorobenzidine)	Isolation of hemoglobin, removal of water, followed by alkaline hydrolysis of adducts; addition of 2,2'-dichloro-benzidine as internal standard; extraction suing toluene containing 5% 2-propanol; derivatization using HFBA.	GC/NCI-MS	<0.1 ng/g (ppb)	65-85% over range 0-150 ng/g. (7% RSD for dichloro-benzidine, 16% RSD for monoacetyl-dichloro-benzidine)	Joppich-Kuhn et al. 1997
Rat hemoglobin adducts (dichloro-benzidine, monoacetyl-dichlorobenzidine)	Isolation of hemoglobin followed by alkaline hydrolysis of adducts, cleanup using C_{18} SPE, addition of internal standard.	HPLC/EC	No data	>90	Birner et al. 1990
Rat hemoglobin adducts (dichloro-benzidine, monoacetyl-dichlorobenzidine)	Isolation of hemglobin followed by alkaline hydrolysis of adducts, addition of recovery standard, cleanup using C_{18} SPE, addition of internal standard.	HPLC/EC	6 ng/g (1 ppb, wt:wt)	92–98	Zwirner-Baier and Neumann 1994
Fish tissue	Digestion with NaOH, extraction with benzene, extraction with dilute H_2SO_4, water removal and volume reduction; GPC cleanup .	GC/HCD (N mode)	<20 ppb	65 (20% RSD)	Diachenko 1979
Rat urine and serum	Addition of internal standard and sodium bicarbonate followed by extraction with diethyl ether; evaporation to dryness and redissolution in toluene.	GC/NPD	5 ng/mL (ppb)	No data	Hoffman and Schmidt 1993

Table 6-1. Analytical Methods for Determining 3,3'-Dichlorobenzidine and Metabolites in Biological Samples (continued)

Sample type	Extraction/cleanup	Detection	Limit of detection	Percent recovery	Reference
Hamster urine (dichlorobenzidine, mono- and di-acetyldichlorobenzidine, conjugates)	Adjustment of pH, extraction with benzene, volume reduction, formation of heptafluorbutyryl derivatives; for conjugates: alkaline hydrolysis of aqueous phase followed by derivatization as above.	GC/ECD	7–48 µg/L	No data	Bowman and Nony 1981; Nony and Bowman 1980; Nony et al. 1980
Urine (dichlorobenzidine, mono- and di-acetyldichlorobenzidine)	Adjustment of pH, extraction with benzene, volume reduction.	HPLC/UV	525 to 660 µg/L	No data	Bowman and Nony 1981; Nony and Bowman 1980; Nony et al. 1980
Urine	Adjustment of pH to 8, adsorption onto C_{18} SPE cartridge and elution with methanol.	HPLC/EC	5 µg/L (ppb)	No data	CPMA 1998
Urine	Adsorption onto XAD-2 resin, elution with acetone followed by clean up using acid-base partitioning and silica gel, formation of penta fluoropropyl derivative.	GC/ECD	≈1 µg/kg (ppb)	41±8	Bowman and Rushing 1981
Urine	Addition of sodium chloride, pH adjustment to 6, extraction with chloroform, extraction of chloroform extract with 3 N HCL; addition of chloramine-T and extraction of colored product into chloroform.	Absorbance at 457 nm	1–2 ppb (µg/L)	68 (4.6% RSD)	Roberts and Rossano 1982

GC = gas chromatography; EC = electrochemical detector; ECD = electron capture detector; HCD = Hall conductivity detector; HFBA = heptafluorobutyric anhydride; HPLC = high performance liquid chromatography; NCI-MS = mass spectrometry in the negative chemical ionization mode; NPD = nitrogen-phosphorus detector; ppb = parts per billion; UV = ultraviolet absorption; SPE = solid phase extraction; wt:wt = weight:weight

have been reported, although the hemoglobin adduct method of Joppich-Kuhn et al. (1997) reported a limit of detection of less than 0.1 ng/g (ppb). These sensitive methods are potentially useful for the assessment of human exposure to 3,3'-dichlorobenzidine.

6.2 ENVIRONMENTAL SAMPLES

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

The determination of 3,3'-dichlorobenzidine in environmental samples is most commonly achieved by GC/mass spectrometry (GC/MS) (Diachenko 1979; EPA 1982b, 1986a, 1984a; Greenberg et al. 1992) and HPLC (Armentrout and Cutie 1980; EPA 1982a; Morales et al. 1981; NIOSH 1994; Riggin and Howard 1979). Sample preparation typically employs liquid-liquid or liquid-solid extractions for water, waste water, soils, sediments, and solid waste. Supercritical fluid extraction has also been shown to provide good recovery of 3,3'-dichlorobenzidine from a spiked, dried soil (Oostdyke et al. 1995). Lopez-Avila et al. (1996) demonstrated that microwave-assisted extraction using a hexane-acetone solvent system gave recoveries from spiked (5 mg/kg), standard soil of 96%. The same solvent system in Soxhlet extraction resulted in only 47% recovery.

Solid phase extraction followed by capillary zone electrophoresis with UV absorbence detection has been shown to be applicable to the isolation and determination of 3,3'-dichlorobenzidine in water at ppm levels (Cavallaro et al. 1995).

For the HPLC determination of 3,3'-dichlorobenzidine 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. HPLC with electrochemical detection is used, providing for a method detection limit of 0.13 μg/L; single operator accuracy and precision for 30 analytes of 5 different types of water samples over a spike range of l-5 μg/L gave an average recovery of 65% and a standard deviation of 9.6% (EPA 1982a). The more complicated the matrix, the more extensive the sample preparation methods generally need to be. In certain circumstances (i.e., relatively clean water samples), water matrices can be introduced directly into the

Table 6-2. Analytical Methods for Determining 3,3'-Dichlorobenzidine in Environmental Samples

Sample type	Extraction/cleanup	Detection	Limit of detection	Percent Recovery	References
Air	Pumping of an aliquot of air through a glass fiber filter, elution with triethylamine in methanol.	HPLC/UV (Method 5509)	0.5 µg/m^3	96	NIOSH 1994
Air (dichlorobenzidine and its salts)	Pumping of an aliquot of air through a glass fiber filter and silica gel, extraction with triethylamine-methanol.	HPLC/UV	3 µg/m^3 for 50 L sample	No data	Morales et al. 1981
Water, wastewater	Extraction with methylene chloride at pH>11 and again at pH<2, removal of water followed by volume reduction.	GC/MS (Standard Method 6410)	16.5 µg/L	110 at 100 µg/L (100 ppb)	Greenberg et al. 1992
Waste water	Extraction with chloroform, solvent exchange to methanol, volume reduction.	HPLC/EC (EPA Method 605)	0.13 µg/L	64 (96% RSD)	EPA 1982a
Water	Adjustment of pH to 6.5–8 followed by filtration and isolation of analyte using SPE with elution using 150 mM phosphoric acid in water-acetone (80:20).	CZE/UV	1.5 mg/L (ppm)	82 (2.4% RSD) at 20 mg/L.	Cavallaro et al. 1995
Water	Adjustment of pH to 11, extraction with solvent such as dichloromethane, removal of water, volume reduction.	GC/MS (EPA Method 625)	16.5 µg/L	143 (145% RSD)	EPA 1982b
Waste water	Addition of isotopically-labeled standard, extraction with methylene chloride at pH 12–13, then at pH <2, removal of water, volume reduction, addition of internal standard.	GC/IDMS (EPA Method 1625)	50 µg/L	106 (25% RSD) at 100 µg/L	EPA 1984a
Waste water	Direct injection into HPLC.	HPLC/UV HPLC/EC	3 ppb (µg/L) with 500 µL injection, EC	87 over range 3 to 12 ppb	Armentrout and Cutie 1980

Table 6-2. Analytical Methods for Determining 3,3'-Dichlorobenzidine in Environmental Samples (continued)

Sample type	Extraction/cleanup	Detection	Limit of detection	Percent Recovery	References
Waste water	Extraction, conversion of 3,3'-dichloro-benzidine to pentafluoropropionamides.	HPLC/EC	0.2 pg		Kawahara et al. 1982
Waste water	Isolation via extraction with chloroform or SPE, addition of or elution with methanol, volume reduction.	HPLC/EC	50–100 ng/L	94 (4% RSD)	Riggin and Howard 1979
Dried soil	Addition of internal standard followed by extraction of soil by SFE with nitrous oxide/methanol/1,6-hexanediamine, expansion of fluid into methylene chloride, volume reduction.	GC/MS	No data	98	Oostdyke et al. 1995
Fish tissue	Digestion with NaOH, extraction with benzene, extraction with dilute H_2SO_4, water removal and volume reduction; GPC cleanup.	GC/HCD (N mode)	<20 ppb	65 (20% RSD)	Diachenko 1979
Waste water, soil, sediment, solid waste	Extraction (liquid-liquid, Soxhlet, sonication) with organic solvent such as dichloromethane, removal of water, volume reduction.	GC/MS (EPA method 8270)	20 µg/L (ppb) for wastewater; 1,300 µg/kg (ppb) for low soil, sediment	110 at 100 µg/L (100 ppb)	EPA 1986a

CZE = capillary zone electrophoresis; EC = electrochemical detector; GC = gas chromatography; HCD = Hall conductivity detector; HPLC = high performance liquid chromatography; IDMS = isotope dilution mass spectrometry; MS = mass spectrometry; RSD = relative standard deviation; SFE = supercritical fluid extraction; SPE = solid phase extraction; UV = ultraviolet absorbance detection

analysis step without prior treatment (Armentrout and Cutie 1980). GC separation methods can be applied also to the extracts obtained for HPLC analyses. Detection of the free amine, in addition to fluorinated derivatives, has been demonstrated by GC methods.

Dichlorobenzidine and its salts are collected from air matrices using adsorption/filtration approaches (Morales et al. 1981; NIOSH 1994) and recovered from the adsorbent using methanol containing a small amount of triethylamine (TEA). The addition of TEA converts any salt to the corresponding amine, thus rendering it soluble in the organic solvent. Limits of detection in the low $\mu g/m^3$ (low to sub-ppb) range have been reported. The compound 4,4'-methylenebis(2-chloroaniline) was reported to interfere with 3,3'-dichlorobenzidine (Morales et al. 1981; NIOSH 1994).

6.3 ADEQUACY OF THE DATABASE

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

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Methods for the determination of 3,3'-dichlorobenzidine in urine and serum have been reported (Birner et al. 1990; Bowman and Nony 1981; Bowman and Rushing 1981; Hoffman and Schmidt 1993; CPMA 1998; Nony and Bowman 1980; Nony et al. 1980; Zwirner-Baier and Neumann 1994). Some of the methods have been shown to be

suitable for the determination of the acetylated metabolites (Bowman and Nony 198 1; Nony and Bowman 1980; Nony et al. 1980). The methods of Birner et al. (1990), Joppich-Kuhn et al. (1997), and Zwirner-Baier and Neumann (1994) permit the analysis of hemoglobin adducts of 3,3'-dichlorobenzidine and its monoacetyl metabolite. Limits of detection for 3,3'-dichlorobenzidine in urine and serum were reported to be as low as 1 to 5 ppb (Bowman and Rushing 1981; Hoffman and Schmidt 1993; Roberts and Rossano 1982), with detectable concentrations of the acetylated metabolites somewhat higher. Most of these studies were performed with samples from rats; the methods should be tested to determine if they are applicable to samples of human origin. In addition, the levels of these biomarkers associated with exposures to 3,3'-dichlorobenzidine of toxicological concern should be defined in order to increase their utility.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods have been described for the determination of 3,3'-dichlorobenzidine in air, with reported limits of detection of 0.5 μg/m^3 (NIOSH 1994) and 3 μg/m^3 (Morales et al. 1981). Methods for the analysis of 3,3'-dichlorobenzidine in water and waste water have also been described, with reported detection limits of 16.5 μg/L (ppb) (EPA 1982b; Greenberg et al. 1992), 50 μg/L (ppb) (EPA 1984a), 3 ppb (Armentrout and Cutie 1980), 0.13 μg/L (ppb) (EPA 1982a), and 50 to 100 ng/L (ppt) (Riggin and Howard 1979). The only method found for 3,3'-dichlorobenzidine in food (fish) reported a limit of detection of less than 20 ppb (Diachenko 1979). It does not appear that additional methods for the determination of 3,3'-dichlorobenzidine in air or water are needed. Additional methods for 3,3'-dichlorobenzidine in foods are needed. If MRLs were established, the needs could be defined more precisely.

6.3.2 Ongoing Studies

No ongoing studies in which new methods for the determination of 3,3'-dichlorobenzidine are being developed were found in a search of the Federal Research in Progress database (FEDRIP 1998).

7. REGULATIONS AND ADVISORIES

The national and state regulations and guidelines pertaining to 3,3'-dichlorobenzidine in air, water, and other media are summarized in Table 7- 1.

There is no oral reference dose (RfD) for 3,3'-dichlorobenzidine. The health effects data for 3,3'-dichlorobenzidine were reviewed by the EPA RfD/RfC Work Group and determined to be inadequate for derivation of an inhalation RfC (IRIS 1998).

The EPA has determined that 3,3'-dichlorobenzidine is a probable human carcinogen, B2 classification (IRIS 1998). The International Agency for Research on Cancer (IARC) has classified 3,3'-dichlorobenzidine as a Group 2B carcinogen-possibly carcinogenic to humans (IARC 1987). The American Conference of Governmental Industrial Hygienists (ACGIH) classifies 3,3'-dichlorobenzidine as A3, which indicates that the chemical is carcinogenic in experimental animals when administered at a relatively high dose (ACGIH 1997). The National Toxicology Program (NTP) of the U.S. Department of Health and Human Services has determined that 3,3'-dichlorobenzidine and its salt may reasonably be expected to be cancer-causing agents (NTP 1998).

3,3'-Dichlorobenzidine is on the list of chemicals subject to the requirements of the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA) (EPA 1988a). Section 3 13 of Title III of EPCRA requires owners and operators of certain facilities that manufacture, import, process, or otherwise use the chemicals on this list to report annually their release of those chemicals to any environmental media (U.S. Congress 1986).

3,3'-Dichlorobenzidine is one of a number of carcinogenic compounds regulated by OSHA. To control exposures to 3,3'-dichlorobenzidine in workplace air and to protect the health of workers, OSHA's regulatory standards provide strict guidelines for manufacturing, processing, repackaging, handling, using, and storing the compound (OSHA 1996). These standards also include the requirements for personal protective equipment, training, labeling, posting, and engineering controls. In addition to engineering controls such as continuous local exhaust ventilation and workplace practices such as full body protective clothing, the employer must maintain medical surveillance records (OSHA 1996). OSHA requires that initial medical screening and regular medical examinations be made available to any employee who is

7. REGULATIONS AND ADVISORIES

exposed to 3,3'-dichlorobenzidine at potentially hazardous levels. The employer must also provide a training program that informs its employees of the carcinogenic hazards of 3,3'-dichlorobenzidine, the nature of the operation involving the chemical that could result in exposure, decontamination procedures,and specific emergency procedures to be used if exposure does occur (OSHA 1996). OSHA also regulates 3,3'-dichlorobenzidine under the Hazard Communication Standard (HCS) and as a chemical hazard in laboratories (NTP 1998). The HCS has established uniform requirements to make sure that the hazards of all chemicals imported into, produced, or used in workplaces are evaluated and that information on the hazards they pose is transmitted to affected employers and exposed employees (OSHA 1998).

EPA regulates 3,3'-dichlorobenzidine under the Clean Air Act (CAA) and has designated 3,3'-dichlorobenzidine as a hazardous air pollutant (HAP) (EPA 1994; U.S. Congress 1990). The major source category for which the national emissions standards for new stationary sources are applicable to 3,3'-dichlorobenzidine emissions is the synthetic organic chemicals manufacturing industry (SOCMI)-equipment leaks (EPA 1994).

3,3'-Dichlorobenzidine is regulated by the Clean Water Effluent Guidelines in Subchapter N of Title 40 of the *Code of Federal Regulations*. Electroplating is the point source category for which 3,3'-dichlorobenzidine is controlled as a total toxic organic (EPA 1981). The point source categories for which 3,3'-dichlorobenzidine has a specific regulatory limitation are steam electric power generation (EPA 1982) and metal finishing (EPA 1983a). The EPA has proposed a reportable quantity of 10 pounds for 3,3'-dichlorobenzidine for its water quality criteria for the protection of human health (IRIS 1998).

The Resource Conservation and Recovery Act (RCRA) identifies 3,3'-dichlorobenzidine as the hazardous constituent in various hazardous wastes. It is the regulated constituent in hazardous wastes assigned the waste code U073 (EPA 1988b).

Under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), owners of vessels or facilities are required to immediately report releases of 3,3'-dichlorobenzidine equal to or greater than the reportable quantity of 1 pound (0.454 kg) (EPA 1985). It is subject to the requirements under the Superfund Amendments and Reauthorization Act (SARA) of 1986 (IRIS 1998).

7. REGULATIONS AND ADVISORIES

Although the Food and Drug Administration (FDA) classifies 3,3'-dichlorobenzidine as a carcinogen, the agency has not enacted regulatory guidelines (NTP 1998) or issued advisories specifically targeting 3,3'-dichlorobenzidine as being a danger in the food supply.

EPA has selected 3,3'-dichlorobenzidine and its mixtures for priority consideration for testing under the Toxic Substances Control Act (TSCA) (IRIS 1998).

Table 7-1. Regulations and Guidelines Applicable to 3,3-Dichlorobenzidine

Agency	Description	Information	References
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenic classification	Group 2B[a]	IARC 1987
WHO	Drinking-water guideline values for health-related organics	None	WHO 1984
NATIONAL			
Regulations:			
a. Air:			
OSHA	Toxic and Hazardous Substances-Carcinogens (4-nitrobiphenyl, etc.)	Yes	29 CFR 1910.1003 OSHA 1996
EPA OAR	Hazardous Air Pollutants	Yes	Clean Air Act Amendment Title III, Section 112 (b) U.S. Congress 1990
	Standards of Performance for New Stationary Sources- Subpart F: National Emission Standards for Organic Hazardous Air Pollution from the Synthetic Organic Chemical Manufacturing Industry (SOCMI)	Yes	40 CFR 63.106 EPA 1994b
b. Water			
EPA OW	EPA Administered Permit Programs: The NPDES- Organic toxic pollutants in each of four fractions in analysis by GC/MS	Yes	40 CFR 122, App. D EPA 1983b
	Methods for organic chemical analysis of municipal and industrial wastewater (Methods 605, 625, and 1625)	Yes	40 CFR 136, App. A EPA 1984b
	Designated as a toxic pollutant under Section 307 (a)(1) of the Federal Water Pollution Control Act	Yes	40 CFR 401.15 EPA 1979b
	General pretreatment regulations for existing and new sources of pollution- List of toxic pollutants	Yes	40 CFR 403, App. B EPA 1986c
	Electroplating Point Source Category- General definition	Yes	40 CFR 413.02 EPA 1981a
	Steam Electric Power Generating Point Source Category Pretreatment standards for new sources (PSNS) Maximum for any time	0 mg/L	40 CFR 423.17 EPA 1982c
	List of 126 priority pollutants	Yes	40 CFR 423, App. A EPA 1982d

Table 7-1. Regulations and Guidelines Applicable to 3,3-Dichlorobenzidine (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
	Metal Finishing Point Source Category Metal finishing subcategory-Definition of total toxic organics (TTO)	>0.01 mg/L	40 CFR 433.11 EPA 1983a
c. Other:			
DOT	Hazardous Substances Other Than Radionuclides: RQ	1 pound (0.454 kg)	49 CFR 172.101, App. A DOT 1990
EPA-OERR	List of Hazardous Substances and Reportable Quantities	1 pound (0.454 kg) (CERCLA statutory)	40 CFR 302.4 EPA 1985
		1 pound (0.454 Kg) (final RQ)	
	Toxic Chemical Release Reporting: Community Right-to-know Specific toxic Chemical Listings	Yes	40 CFR 372.65 EPA 1988a
EPA-OSW	Criteria for Municipal Solid Waste Landfills		
	List of hazardous inorganic and organic constituents	Yes	40 CFR 258, App. II EPA 1991a
	Identification and Listing of Hazardous Wastes Subpart D: Lists of Hazardous Wastes Discarded commercial products, off-specification species, container residues, and spill residues (U073)	Yes	40 CFR 261.33 EPA 1980a
	Hazardous Constituents	U073	40 CFR 261, App. VIII EPA 1988b
	Standards for Owners and Operators of Hazardous Waste Treatment, Storage, and Disposal Facilities		
	Ground-water monitoring list	Yes	40 CFR 264, App. IX EPA 1987a
	Land Disposal Restrictions- Subpart B: Schedule for land disposal prohibition and establishment of treatment standards	Yes	40 CFR 268.11 EPA 1986b

Table 7-1. Regulations and Guidelines Applicable to 3,3-Dichlorobenzidine (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
	Subpart C: Prohibitions on land disposal	Yes	40 CFR 268.35 EPA 1990b
	Subpart D: Treatment Standards Treatment standards for hazardous waste and Universal treatment standards--Technical amendment of final rule (40 CFR 268.40--waste code U073)	WETOX or CHOXD; CARBN or INCIN	62 FR 7502 EPA 1997
	List of halogenated organic compounds regulated under 268.32	Yes	40 CFR 268, App. III EPA 1987b
	Organometallic lab packs	Yes	40 CFR 268, App. IV EPA 1991b
Guidelines:			
a: Air:			
NIOSH	Recommended Exposure Limit for Occupation Exposure	Use 29 CFR 1910.1007	NIOSH 1997
b. Water			
EPA OW	Ambient Water Quality Criteria for Human Health		IRIS 1998
	water and fish	0.01 µg/L	
	fish only	0.02 µg/L	
c. Other:			
ACGIH	Cancer classification	A3[b]	ACGIH 1997
EPA OWRS	Cancer classification	B2[c]	IRIS 1998
	Drinking Water Concentrations at Specified Risk Levels		
	E-4 (1 in 10,000)	8.0 µg/L	
	E-5 (1 in 100,000)	0.8 µg/L	
	E-6 (1 in 1,000,000)	0.08 µg/L	
NTP	Cancer classification	Reasonably anticipated to be a human carcinogen	NTP 1998
STATE			
Regulations and Guidelines:			
a. Air:	Average Acceptable Ambient Air Concentrations		
MI	Annual	2.00×10^{-3} µg/m^3	NATICH 1992
ND	Not specified	0.0 BACT	
NY	1 Year	1.00 µg/m^3	
OK	Not specified	0.0	

Table 7-1. Regulations and Guidelines Applicable to 3,3-Dichlorobenzidine (continued)

Agency	Description	Information	References
STATE (cont.)			
PA-Philadelphia	Not specified	0.0	
RI	Annual	$2.0x10^{-3}$ µg/m^3	
SC	24 hours	$1.50x10^{-1}$ µg/m^3	
VA	24 hours	0.0 µg/m^3	
b. Water			
	Water Quality Criteria: Human Health		
AZ	Drinking water (guideline)	0.020 µg/L	FSTRAC 1990
FL	Domestic/drinking	20 µg/L	Sittig 1994
KS	Drinking water (guideline)	0.21 µg/L	FSTRAC 1990
MA	Domestic/drinking	80 µg/L	Sittig 1994
MI	Domestic/drinking	0.077 µg/L	
MN	Drinking water (guideline)	0.21 µg/L	FSTRAC 1990
NH	Drinking water (guideline)	0.02 µg/L	
NJ	Domestic/drinking	60 µg/L	Sittig 1994
OR	Domestic/drinking	0.2 µg/L	

[a] Group 2B defines the agent as possibly carcinogenic to humans. The category is generally used for agents for which there is limited evidence in humans in the absence of sufficient evidence in experimental animals.

[b] Cancer classification A3 indicates that the agent is carcinogenic in experimental animals at a relatively high dose.

[c] Group B defines the substance as a probable human carcinogen where there is limited evidence in epidemiologic studies (Group B1) and/or sufficient evidence from animal studies.

ACGIH = American Conference of Governmental Industrial Hygienists; BACT = Best Available Control Technology; CARBN = carbon adsorption; CHOXD = chemical or electrolytic oxidation; DOT = Department of Transportation; EPA = Environmental Protection Agency; FSTRAC = Federal State Toxicology and Regulatory Alliance committee; GC/MS = Gas Chromatography/Mass Spectroscopy; IARC = International Agency for Research on Cancer; INCIN = incineration; NATICH = Nation Air Toxics Information Clearinghouse; NIOSH = National Institute of Occupational Safety and Health; NPDES = National Pollution Discharge Elimination System; NTP = National Toxicology Program; OAR = Office of Air and Radiation; OERR = Office of Emergency and Remedial Response; OSHA = Occupational Safety and Health Administration; OSW = Office of Solid Wastes; PSNS = Performance Standards for New Sources; RQ = Reportable Quantities; SOCMI = Synthetic Organic Chemicals Manufacturing Industry; TTO = Total Toxic Organics; WETOX = wet air oxidation; WHO = World Health Organization

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9. GLOSSARY

Absorption-The taking up of liquids by solids, or of gases by solids or liquids.

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

Adsorption-The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})-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.

Benchmark Dose (BMD) -is usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD_{10} would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

Benchmark Dose Model-is a statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

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 period.

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

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

Carcinogen-A chemical capable of inducing cancer.

Case-Control Study-A type of epidemiological study which examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

Case Report-describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research but are not actual research studies.

9. GLOSSARY

Case Series-describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research but are not actual research studies.

Ceiling Value-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.

Cohort Study-A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

Cross-sectional Study-A type of epidemiological study of a group or groups which examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

Data Needs-substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

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.

Dose-Response Relationship-The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

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 occurs. The terms, as used here, include malformations and variations, altered growth, and in utero death.

Environmental Protection Agency (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. Epidemiology-refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Genotoxicity-a specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life-a measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

9. GLOSSARY

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

Incidence--The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

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

Immunological Effects-are functional changes in the immune response.

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

Lethal Concentratioq$_{(50)}$ (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$_{(50)}$ (LD_{50}) -The lowest dose of a chemical introduced by a route other than inhalation that has been reported 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.

Lethal Tlme$_{(50)}$ (LT_{50})-A calculated period of time within which a specific concentration of a chemical isexpected to cause death in 50% of a defined experimental animal population.

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

Lymphoreticular Effects-represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus. Malformations-Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL) -An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

9. GLOSSARY

Modifying Factor (MF)-A value (greater than zero) that is applied to the derivation of a minimal risk level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity-State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality-Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen-A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy-The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

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

No-Observed-Adverse-Effect Level (NOAEL)-The dose of a chemical at which there were 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 (K_{ow})-The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio-a means of measuring the association between an exposure (such as toxic substances and a disease or condition) which represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed.

Organophosphate or Organophosphorus Compound-a phosphorus containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

Permissible Exposure Limit (PEL)-An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an &hour shift of a 40 hour workweek.

Pesticide-general classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

Pharmacokinetics-is the science of quantitatively predicting the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism and excretion of chemicals by the body.

9. GLOSSARY

Pharmacokinetic Model - is a set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments which, in general, do not represent real, identifiable anatomic regions of the body whereby the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model - is a type of physiologically-based doseresponse model which quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model - is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates and, possibly membrane permeabilities. The models also utilize biochemical information 4such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence-The number of cases of a disease or condition in a population at one point in time.

Prospective Study--a type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

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 μg/L for water, μg/kg/day for food, and μg/m^3 for air).

Recommended Exposure Limit (REL)-A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentrations for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)-An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3 or ppm.

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 No-Observed-Adverse-Effect Level (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.

9. GLOSSARY

Reportable Quantity (RQ)-The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour 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.

Retrospective Study-A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to casual factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk-the possibility or chance that some adverse effect will result from a given exposure to a chemical.

Risk Factor-An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic, that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio--The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed.

Short-Term Exposure Limit (STEL)-The American Conference of Governmental Industrial Hygienists (ACGIH) 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 Threshold Limit Value - Time Weighted Average (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.

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

Threshold Limit Value (TLV)-An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

Time-Weighted Average (TWA)-An allowable exposure concentration averaged over a normal &hour workday or 40-hour workweek. .

Toxic Dose$_{(50)}$ (TD$_{50}$) - 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.

9. GLOSSARY

Toxicokinetic-The study of the absorption, distribution and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)-A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) 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 human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using Lowest-Observed-Adverse-Effect Level (LOAEL) data rather than No-Observed-Adverse-Effect Level (NOAEL) data. A default for each individual W is 10; if complete certainty in data exists, a value of one can be used; however a reduced UF of three may be used on a case-by-case basis, three being the approximate logarithmic average of 10and 1.

Xenobiotic-any chemical that is foreign to the biological system.

APPENDIX A

ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99-4991, requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (l-14 days), intermediate (15-364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

APPENDIX A

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as a hundredfold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agencywide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road, Mailstop E-29, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

No MRLs were derived for 3,3’-dichlorobenzidine.

APPENDIX B

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical. The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Tables and Figures for Levels of Significant Exposure (LSE)

Tables (2-1, 2-2, and 2-3) and figures (2-l and 2-2) are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, minimal risk levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse- Effect Levels (NOAELs), Lowest-Observed-Adverse-Effect Levels (LOAELs), or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 2-l and Figure 2-l are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 2-1

(1) Route of Exposure One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data exists, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 2-1, 2-2, and 2-3, respectively). LSE figures are limited to the inhalation (LSE Figure 2-l) and oral (LSE Figure 2-2) routes. Not all substances will have data on each route of exposure and will not therefore have all five of the tables and figures.

APPENDIX B

(2) Exposure Period Three exposure periods - acute (less than 15 days), intermediate (15-364 days), and chronic (365 days or more) are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the 2 "18r" data points in Figure 2-l).

(5) Species The test species, whether animal or human, are identified in this column. Section 2.5, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 2.3, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration The duration of the study and the weekly and daily exposure regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to 1,1,2,2-tetrachloroethane via inhalation for 6 hours per day, 5 days per week, for 3 weeks. For a more complete review of the dosing regimen refer to the appropriate sections of the text or the original reference paper, i.e., Nitschke et al. 1981.

(7) System This column further defines the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, 1 systemic effect (respiratory) was investigated.

(8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference The complete reference citation is given in chapter 8 of the profile.

APPENDIX B

(11) CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Figure 2-1

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14) Health Effect These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL In this example, 18r NOAEL is the critical endpoint for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates to a NOAEL for the test species-rat. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL Key number 38r is 1 of 3 studies for which Cancer Effect Levels were derived. The diamond symbol refers to a Cancer Effect Level for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure The Key explains the abbreviations and symbols used in the figure.

SAMPLE

1 →

TABLE 2-1. Levels of Significant Exposure to [Chemical x] – Inhalation

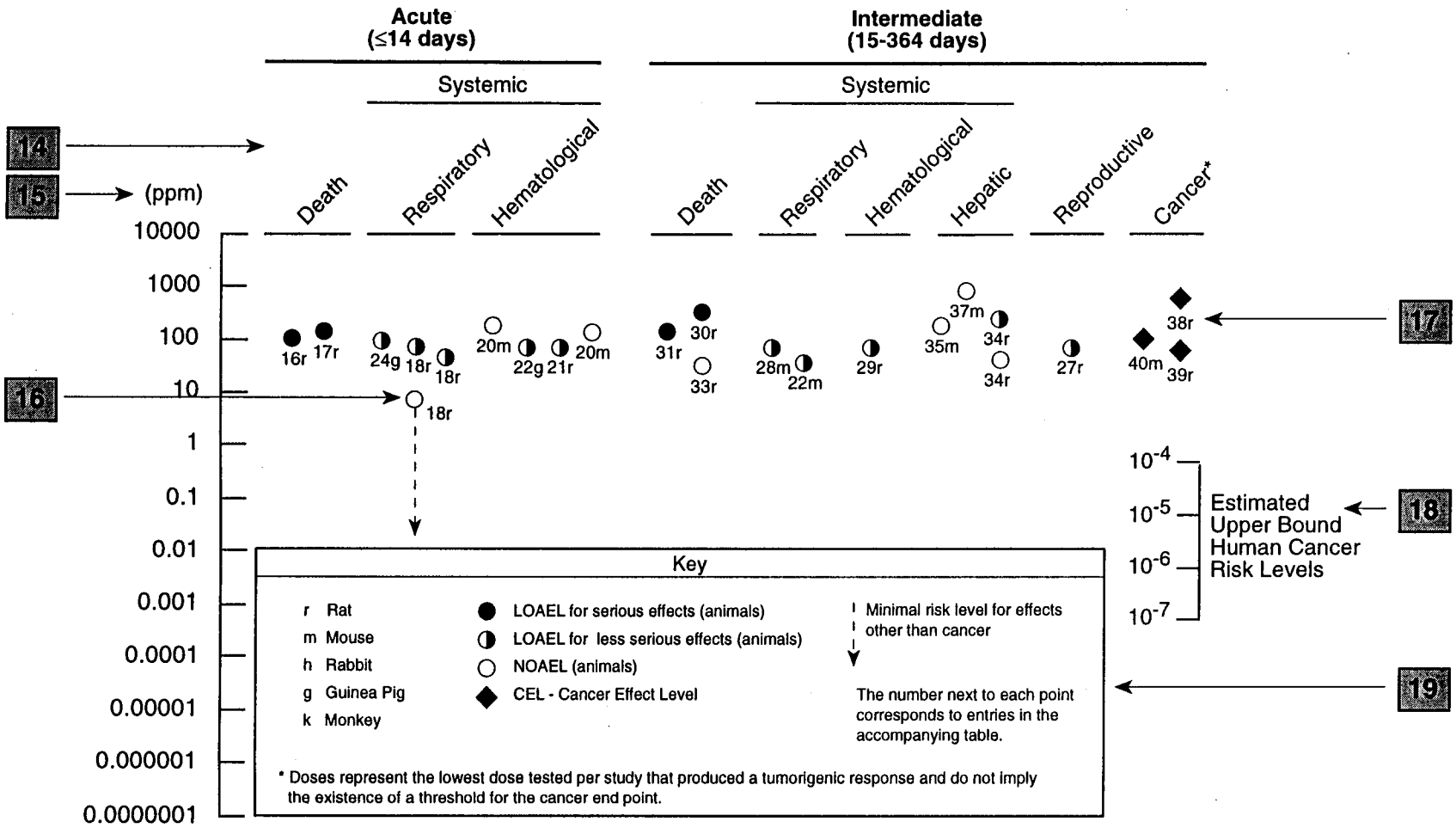
Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 → INTERMEDIATE EXPOSURE							
	5	6	7	8	9		10
3 → Systemic	↓	↓	↓	↓	↓		↓
4 → 18	Rat	13 wk 5d/wk 6hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
					11		
Cancer					↓		
38	Rat	18 mo 5d/wk 7hr/d			20	(CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5d/wk 6hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5d/wk 6hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

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

12 → [b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5 x 10 ppm^3; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

13 → **Figure 2-1. Levels of Significant Exposure to [Chemical X] – Inhalation**



APPENDIX B

Chapter 2 (Section 2.5)

Relevance to Public Health

The Relevance to Public Health section provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions.

1. What effects are known to occur in humans?

2 . What effects observed in animals are likely to be of concern to humans?

3 . What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The section covers end points in the same order they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this section. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal risk levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, we have derived minimal risk levels (MRLs) for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action; but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2.5, "Relevance to Public Health," contains basic information known about the substance. Other sections such as 2.8, "Interactions with Other Substances," and 2.9, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses for lifetime exposure (RfDs).

APPENDIX B

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgment, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

APPENDIX C

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism, and Excretion
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	Best Available Technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BSC	Board of Scientific Counselors
C	Centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	Cancer Effect Level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CNS	central nervous system
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
d	day
Derm	dermal
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation
DOT/UN/ NA/IMCO	Department of Transportation/United Nations/ North America/International Maritime Dangerous Goods Code
DWEL	Drinking Water Exposure Level

APPENDIX C

ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
ft	foot
FR	*Federal Register*
g	gram
GC	gas chromatography
Gd	gestational day
gen	generation
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
hr	hour
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IDLH	Immediately Dangerous to Life and Health
IARC	International Agency for Research on Cancer
ILO	International Labor Organization
in	inch
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{Lo}	lethal concentration, low
LC_{50}	lethal concentration, 50% kill
LD_{Lo}	lethal dose, low
LD_{50}	lethal dose, 50% kill
LT_{50}	lethal time, 50% kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter
MA	*trans,trans*-muconic acid
MAL	Maximum Allowable Level
mCi	millicurie

APPENDIX C

MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mm Hg	millimeters of mercury
mmol	millimole
mo	month
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCI	National Cancer Institute
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NFPA	National Fire Protection Association
ng	nanogram
NLM	National Library of Medicine
nm	nanometer
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances

OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	Polycyclic Aromatic Hydrocarbon
PBPD	Physiologically Based Pharmacodynamic
PBPK	Physiologically Based Pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
PID	photo ionization detector
pg	picogram
pmol	picomole
PHS	Public Health Service
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	Pretreatment Standards for New Sources
REL	recommended exposure level/limit
RfC	Reference Concentration
RfD	Reference Dose
RNA	ribonucleic acid
RTECS	Registry of Toxic Effects of Chemical Substances
RQ	Reportable Quantity
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
sec	second
SIC	Standard Industrial Classification
SIM	selected ion monitoring
SMCL	Secondary Maximum Contaminant Level
SMR	standard mortality ratio
SNARL	Suggested No Adverse Response Level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short-term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	Total Organic Compound
TPQ	Threshold Planning Quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TRI	Toxics Release Inventory
TWA	time-weighted average
U.S.	United States
UF	uncertainty factor
VOC	Volatile Organic Compound
yr	year
WHO	World Health Organization
wk	week

APPENDIX C

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

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