

**TOXICOLOGICAL PROFILE FOR  
BENZIDINE**

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

September 2001

## **DISCLAIMER**

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

## **UPDATE STATEMENT**

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:

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



### 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.

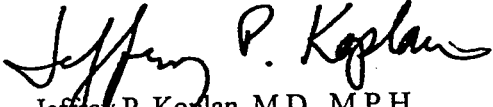
The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels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

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 prepared 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, 1990 (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.

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### *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: Relevance to Public Health:** The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

**Chapter 3: 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:

<b>Section 1.6</b>	<b>How Can (Chemical X) Affect Children?</b>
<b>Section 1.7</b>	<b>How Can Families Reduce the Risk of Exposure to (Chemical X)?</b>
<b>Section 3.7</b>	<b>Children's Susceptibility</b>
<b>Section 6.6</b>	<b>Exposures of Children</b>

**Other Sections of Interest:**

<b>Section 3.8</b>	<b>Biomarkers of Exposure and Effect</b>
<b>Section 3.11</b>	<b>Methods for Reducing Toxic Effects</b>

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### *ATSDR Information Center*

**Phone:** 1-888-42-ATSDR or (404) 639-6357    **Fax:** (404) 639-6359  
**E-mail:** [atsdric@cdc.gov](mailto:atsdric@cdc.gov)    **Internet:** <http://www.atsdr.cdc.gov>

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—*Medical Management 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.

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### ***Other Agencies and Organizations***

*The National Center for 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 20201 • 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.

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### ***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, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: [AOEC@AOEC.ORG](mailto:AOEC@AOEC.ORG) • Web Page: <http://www.aoec.org/>.

*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 benzidine. The panel consisted of the following members:

1. Dr. James Collins, Director of Epidemiology, Monsanto, St. Louis, MO;
2. Dr. Arthur Gregory, Private Consultant, Techt Enterprises, Sterling, VA;
3. Dr. Linvall DePass, Department Head-Toxicology, Roche Bioscience, Palo Alto, CA; and
4. Dr. Edmond LaVoie, Professor and Chairman, Pharmaceutical Chemistry, Piscataway, NJ.

These experts collectively have knowledge of benzidine'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(i)(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.



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## 1. PUBLIC HEALTH STATEMENT

This public health statement tells you about benzidine 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. Benzidine has been found in at least 28 of the 1,585 current or former NPL sites. However, the total number of NPL sites evaluated for benzidine is not known. As more sites are evaluated, the sites at which benzidine is found may increase. This information is important because exposure to benzidine 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 benzidine, 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.

### 1.1 WHAT IS BENZIDINE?

Benzidine is a manufactured chemical that does not occur naturally. It is a crystalline (sandy or sugar-like) solid that may be grayish-yellow, white, or reddish-gray. It will evaporate slowly from water and soil. Its flammability, smell, and taste have not been described. Benzidine also has other names, such as 4,4'-diphenylenediamine or Fast Corinth Base B (a registered trade name). In the environment, benzidine is found in either its "free" state (as an organic base), or as a salt (for example, benzidine dihydrochloride or benzidine sulfate). In air, benzidine is found attached to suspended particles or as a vapor.

## 1. PUBLIC HEALTH STATEMENT

In the past, industry used large amounts of benzidine to produce dyes for cloth, paper, and leather. However, it has not been made for sale in the United States since the mid-1970s. Major U.S. dye companies no longer make benzidine-based dyes. Benzidine is no longer used in medical laboratories or in the rubber and plastics industries. However, small amounts of benzidine may still be manufactured or imported for scientific research in laboratories or for other specialized uses. Some benzidine-based dyes (or products dyed with them) may also still be brought into the United States.

See Chapters 4 and 5 for more information on the properties and uses of benzidine.

**1.2 WHAT HAPPENS TO BENZIDINE WHEN IT ENTERS THE ENVIRONMENT?**

In the past, benzidine entered the environment largely when it was being made or used to produce dyes. Industry released it to waterways in the form of liquids and sludges, and transported benzidine-containing solids to storage or waste sites. Benzidine was sometimes accidentally spilled, and it was released to the air as dust or fumes. For the most part, companies no longer make or use benzidine, and the government strictly regulates these activities. Today, most benzidine still entering the environment probably comes from waste sites where it had been disposed. Some may also come from the chemical or biological breakdown of benzidine-based dyes, or from other dyes where it may exist as an impurity.

Only very small amounts of free benzidine will dissolve in water at moderate environmental temperatures. When released into waterways, it will sink and become part of the bottom sludge. Benzidine salts can dissolve more easily in water than free benzidine. Only a very small portion of dissolved benzidine will pass into the air. Benzidine exists in the air as very small particles or as a vapor, which may be brought back to the earth's surface by rain or gravity. In soil, most benzidine is likely to be strongly attached to soil particles, so it does not easily pass into underground water.

## 1. PUBLIC HEALTH STATEMENT

Benzidine can slowly be destroyed by certain other chemicals, light, and some microorganisms (for example, bacteria). Certain fish, snails, algae, and other forms of water life may take up and store very small amounts of benzidine, but accumulation in the food chain is unlikely.

See Chapters 5 and 6 for more information about how benzidine behaves in the environment.

**1.3 HOW MIGHT I BE EXPOSED TO BENZIDINE?**

The general population is not likely to be exposed to benzidine through contaminated air, water, soil, or food. Benzidine is a manufactured chemical that does not occur naturally in the environment. Today, U.S. industry makes and uses very little (if any) benzidine, and no releases to air, water, or soil are reported on the Toxic Release Inventory (TRI). Only rarely has benzidine been detected in areas other than waste sites, and it has not been found in food. Some dyes used to color foods or drinks may contain impurities that can be broken down to benzidine once inside the body.

If you live near a hazardous waste site, you could be exposed to benzidine by drinking contaminated water or by breathing or swallowing contaminated dust and soil. Benzidine can also enter the body by passing through the skin.

Some quantities of dyes made from benzidine may still be brought into the United States. These may contain small amounts of benzidine as a contaminant, or chemicals that may be broken down in the body to benzidine. If you use such dyes to dye paper, cloth, leather, or other materials, you may be exposed by breathing or swallowing dust, or through skin contact with dust. You may be exposed in a similar way if you work at or near hazardous waste sites.

See Chapter 6 for more information on how you can be exposed to benzidine and benzidine-based dyes.

## 1. PUBLIC HEALTH STATEMENT

**1.4 HOW CAN BENZIDINE ENTER AND LEAVE MY BODY?**

Benzidine can enter your body if you breathe air that has small particles of benzidine or dust to which benzidine is attached. It can also enter your body if you drink water or eat food that has become contaminated with benzidine. If your skin comes in contact with benzidine, it could also enter your body. Generally, it will take only a few hours for most of the benzidine to get into your body through the lungs and intestines. It may take several days for most of the benzidine to pass through your skin. Breathing, eating, or drinking benzidine-based dyes may also expose you to benzidine. Your intestines contain bacteria that can break down these dyes into benzidine.

Once in your body, only a small portion of benzidine will leave as waste in your urine and feces. Your body will change most of the benzidine into many different chemical forms (called metabolites), which dissolve readily in your bodily fluids and are easy for your body to remove. Some of these changed forms of benzidine appear to cause many of the chemical's harmful effects. Studies show that after benzidine has entered your body, most of it (and its changed forms) will be removed within a week.

See Chapter 3 for more information on how benzidine can enter, be changed in, and leave your body.

**1.5 HOW CAN BENZIDINE AFFECT MY HEALTH?**

Very little information is available on the noncancer health effects that may be caused by exposure to benzidine. Benzidine contact with your skin could possibly cause a skin allergy. Except for the cancer discussed next, benzidine has not been definitely shown to cause major adverse health effects in humans.

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.



## 1. PUBLIC HEALTH STATEMENT

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.

Benzidine can cause cancer. This has been shown in studies of workers who were exposed for many years to levels much higher than the general population would experience. It is important to note that most of the workers did not develop cancer, even after such high exposures. When cancer does occur, most often it is cancer of the urinary bladder. Some evidence suggests that other organs, such as the stomach, kidney, brain, mouth, esophagus, liver, gallbladder, bile duct, and pancreas, may also be affected. Experiments with animals have also shown benzidine to be a carcinogen (a cancer causing substance). The Department of Health and Human Services (DHHS), the International Agency for Research on Cancer (IARC), and the EPA have determined that benzidine is a human carcinogen. In addition, dyes made from benzidine, such as Direct Blue 6, Direct Black 38, and Direct Brown 95, have been shown to cause cancer in animals, and there is some evidence that they may cause bladder cancer in humans. DHHS has determined that Direct Black 38 and Direct Blue 6 cause cancer in animals, and IARC has also determined that Direct Black 38, Direct Blue 6, and Direct Brown 95 cause cancer in animals.

See Chapter 3 for more information on the health effects of benzidine.

## **1.6 HOW CAN BENZIDINE 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.

## 1. PUBLIC HEALTH STATEMENT

Children might be exposed to benzidine if they eat small amounts of soil contaminated with benzidine. However, studies suggest that it is difficult to release benzidine once it becomes attached to most types of soils. Exposure by contaminated soil may occur if the children live in an area near a source of the chemical (such as a hazardous waste site that contains benzidine).

There are no studies on health effects in children exposed to benzidine. There is no information on whether benzidine causes birth defects in children. It is unknown whether birth defects would occur in the newborn babies of animals that breathed or ate benzidine, or had it on their skin while they were pregnant.

There is no information to determine whether children differ from adults in their sensitivity to the health effects of benzidine. There is indirect evidence that benzidine or its breakdown products can cross the placenta, but it is not known whether it can be transferred to the young through the mother's milk. More information regarding children's health and benzidine can be found in Section 3.7.

### **1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO BENZIDINE?**

If your doctor finds that you have been exposed to significant amounts of benzidine, ask whether your children might also be exposed. Your doctor might need to ask your state health department to investigate.

Benzidine has no agricultural or food chemical uses, so exposure to it by eating contaminated food is not likely. Impurities in certain food dyes can be transformed inside the body to benzidine. Children may be exposed to benzidine if they eat small amounts of soil contaminated with benzidine. Children should be prevented from eating soil; make sure they wash their hands frequently, and before eating. Discourage your children from putting their hands in their mouths or from doing other hand-to-mouth activities.

More information regarding exposure to benzidine can be found in Sections 6.5, 6.6, and 6.7.

## 1. PUBLIC HEALTH STATEMENT

**1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO BENZIDINE?**

Several tests have been developed to help determine whether you have been exposed to benzidine. Although these tests must be performed by experts in special laboratories, your doctor can collect the blood or urine samples and send them to an appropriate testing facility. Benzidine and its breakdown products can be detected in your urine, but only within about 2 weeks after your last exposure. Benzidine and some of its changed forms will bind to proteins within your red blood cells, and this can be detected for up to 4 months after your last exposure. Benzidine in some of its forms can bind to the DNA found in most of your cells. There are extremely sensitive tests that can detect such binding; scientists continue to investigate whether these tests can detect benzidine only or other similar chemicals too. None of these tests, however, can predict whether harmful effects will occur later.

See Chapters 3 and 7 for more information on tests that can help determine whether you have been exposed to benzidine.

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

The federal government develops regulations and recommendations to protect public health. Regulations can be enforced by law. Federal agencies that develop regulations for toxic substances include the Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA), and the 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

## 1. PUBLIC HEALTH STATEMENT

different exposure times (an 8-hour workday or a 24-hour day), the use of different animal studies, or other factors.

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 benzidine include the following:

Because benzidine can cause cancer, the EPA has issued regulations that list it as a "priority" chemical, subject to rigid inspection and control. EPA allows 0.10 parts of benzidine per million parts of waste (0.10 ppm) that is brought to waste disposal sites. EPA also requires that any release of one pound or more of benzidine or its salts to the environment must be reported to the federal government's National Response Center. EPA's Office of Water also has water quality guidelines to protect human health. These guidelines suggest that benzidine concentration limits should be maintained at less than 1 part benzidine in a trillion parts of water (ppt). Although zero benzidine is preferred, lifetime exposure to these concentrations is estimated to result in no more than one additional case of cancer in a million persons exposed.

OSHA considers benzidine to be a carcinogen, and has issued regulations to reduce the risk of exposure in any workplace in which it might still be found. These regulations include entry controls, housekeeping and disposal rules, other rules on operating and handling procedures, and requirements that employers make showers and dressing rooms available.

NIOSH recommends that worker exposure to benzidine-based dyes be kept to the lowest feasible concentration, and it considers benzidine to be an occupational carcinogen. EPA's Office of Water has set a discharge limit for benzidine-based dye applicators of 10 micrograms per liter (10 µg/L) (one µg is one millionth of a gram) over any calendar month or not more than 25 µg/L in any working day.

FDA allows a maximum of 1 part of benzidine per billion parts (ppb) of some color additives for foods.

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More information on regulations and advisories is presented in Chapter 8.

**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: (888) 42-ATSDR (1-888-422-8737)  
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 22161  
Phone: (800) 553-6847 or (703) 605-6000



## 2. RELEVANCE TO PUBLIC HEALTH

### 2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO BENZIDINE IN THE UNITED STATES

Benzidine is a crystalline solid that may be grayish-yellow, white, or reddish-gray in color. It is a synthetic chemical with low volatility and is moderately soluble in water and organic solvents. In the past, benzidine was primarily used for the manufacture of dyes, especially azo dyes in the leather, textile, and paper industries. Benzidine is no longer produced for commercial sale or imported in the United States. In 1973, OSHA regulations effectively banned U.S. production of benzidine. Any benzidine production must be for captive consumption (in-house use by the producer only), and it must be maintained in closed systems under stringent workplace controls.

Generally, ATSDR believes that the primary route of human exposure to benzidine at hazardous waste sites is by ingestion of contaminated media, and to a much lesser extent, by dermal contact with contaminated soil. Benzidine has been found in at least 28 of the 1,585 current or former NPL sites. It was identified in surface water collected at 5 sites, groundwater collected at 10 sites, soil samples collected from 9 sites, and sediment samples collected from 4 of these 28 NPL hazardous waste sites. Benzidine was not detected in air at any of the NPL hazardous waste sites. No data are available on the levels of benzidine in body tissues or fluids for those living near hazardous waste sites or for the general population, but as previously indicated, since benzidine is no longer produced or used commercially in the United States, occupational exposures and exposures to the general population are expected to be low. Exposure to benzidine through most food products is highly unlikely; however, impurities found in certain food dyes can be metabolized to benzidine once inside the body. No data were located regarding dietary intake of benzidine. There are no studies that specifically addressed the health effects of exposure to benzidine in children or immature animals; therefore, it is unknown whether children differ from adults in their susceptibility to health effects from benzidine. There do not appear to be unique exposure pathways to benzidine for children.

## 2. RELEVANCE TO PUBLIC HEALTH

**2.2 SUMMARY OF HEALTH EFFECTS**

Information on the toxicity of benzidine in humans is derived mainly from studies of individuals exposed in the workplace. Exposures in such settings have been assumed to have been mainly via inhalation and dermal contact. The toxicity of benzidine has been investigated in animals by the oral route. Cancer is the principal and best documented toxic effect of benzidine in both humans and animals. Humans and dogs develop primarily cancer of the bladder, whereas rodents develop primarily liver cancer. Relatively little information is available on the noncancer effects of benzidine. Dermal and immunological effects have been reported in workers exposed to benzidine, and animal studies have reported hepatic, renal, immunological, and neurological effects. The immunological alterations in the workers were subtle, and none of the subjects exhibited signs of infection at the time of the study. Few studies were located regarding reproductive or developmental toxicity of benzidine in humans or animals. A single, somewhat limited epidemiological study failed to detect any evidence of elevated rates for birth defects in residents living near a Superfund site contaminated with benzidine. However, the extent of actual exposure (if any) to benzidine was not determined. The only animal data, from an acute intraperitoneal study in mice, did not reveal any adverse effects of benzidine on sperm morphology. As detailed in Section 2.3 and Chapter 3, the overall quality of the noncancer database is inadequate and the relevance of these observations to human health is unclear. Therefore, the section below focuses only on the health effect of major concern for benzidine, cancer.

**Cancer.** Epidemiological evidence is currently sufficient to qualitatively establish that benzidine is a human bladder carcinogen following long-term occupational exposure. Some studies have observed benzidine-associated increased risks for cancer at one or more other human tissue sites as well (i.e., stomach, kidney, central nervous system, oral cavity, larynx, esophagus, liver, gallbladder, bile duct, and pancreas). The evidence for benzidine-induced cancer in sites other than the bladder, however, is not as strong as for bladder cancer. Reasons for this include the small number of cases among the populations studied, which diminishes the power of the statistical analyses, and inconsistency between studies regarding tumor sites. The occurrence of liver and kidney cancer has some biological plausibility based on what is known about the mechanisms of benzidine carcinogenicity (see Section 3.5.2). The exposure pathways in the workplace are not known with certainty, but most probably involve a mixture of inhalation and dermal routes. While no studies were located on cancer in humans after oral exposure to benzidine itself, oral exposure to benzidine-based dyes has been reported to increase the risk of bladder cancer in Japanese kimono painters.



## 2. RELEVANCE TO PUBLIC HEALTH

The findings of cancer in humans are supported by observations that inhalation or oral exposure to benzidine can induce mammary and liver tumors and leukemia in rats, and that oral exposure can induce liver and bile duct cancer in hamsters; liver, Harderian gland, uterine, lung, and reticulum cell cancers in mice; and bladder cancer in dogs. Although particular target organs seem species-specific and probably related to differences in metabolism, benzidine appears capable of inducing cancer in most species by most routes of exposure. There is no evidence to suggest that the carcinogenicity of benzidine is route-dependent.

The mechanism of carcinogenicity of benzidine has been studied extensively and is thought to involve the formation of reactive intermediates as a result of metabolic transformations of benzidine. These reactive intermediates are thought to produce DNA adducts, which may initiate carcinogenesis by producing mutations that become fixed before DNA can be repaired. For further details on the mechanism of benzidine carcinogenicity, see Section 3.5.2, Mechanisms of Toxicity. Susceptibility to bladder cancer has been linked to slow acetylator type of the NAT2 N-acetyltransferase gene. Research conducted in recent years, however, has shown that slow acetylators are not at increased risk for bladder cancer, relative to fast acetylators (see Section 3.10 for further details).

The Department of Health and Human Services (DHHS) and the EPA have determined that benzidine is a known human carcinogen. The International Agency for Research on Cancer (IARC) has determined that benzidine is carcinogenic to humans.

### 2.3 MINIMAL RISK LEVELS

#### *Inhalation MRLs*

No inhalation MRLs were derived for benzidine because of lack of sufficient inhalation data for any duration category. The only noncancer information regarding inhalation exposure in humans comes from epidemiological reports of altered natural killer cell function and of changes in values of T lymphocyte subpopulations in exposed workers. The utilization of these studies for MRL development is limited by exposure to other aromatic amines and lack of exposure concentration data. In animals, the only study providing quantitative inhalation data focused on carcinogenic effects with no data on noncancer end points.

## 2. RELEVANCE TO PUBLIC HEALTH

*Oral MRLs*

No oral MRLs were derived for benzidine because of inadequate data, as detailed below. Acute studies by the oral route were limited to studies examining mortality in animals and to a study that reported potential immunotoxicity in mice exposed to an unspecified dose of benzidine. Some intermediate-duration studies were available that described cardiovascular, hepatic, renal, hematological, immunological, or weight-loss effects. However, those studies are inadequate for derivation of an intermediate oral MRL because they did not provide quantitative data; most had inadequate design and/or incomplete reporting of the results (i.e., no controls, dose at which the effect was seen was not identified, only one dose level was tested, high mortality). Several studies provided data on effects after chronic-duration exposure in animals, but were considered inadequate for derivation of a chronic oral MRL. For example, brain vacuolization, a serious effect that precludes its use for MRL derivation, was seen in mice exposed to approximately 1.8 mg/kg/day, the lowest dose tested in another study. Weight loss, liver foci (which may represent preneoplastic lesions), hemosiderin pigmentation of the spleen, and bile duct hyperplasia have been reported in mice after chronic-duration oral exposure to benzidine doses between approximately 2.5 and 11 mg/kg/day. All of these effects occurred at dose levels higher than the one that caused brain vacuolization. A marginally significant ( $p < 0.10$ ) increase in proteinaceous kidney casts in female mice was reported, but not the specific dose at which the effect was observed. Liver cirrhosis was observed in several rabbits orally exposed for up to 3.5 years; however, only a summary of the data was presented. Recurring renal cystitis was the only treatment-related systemic effect observed in seven dogs exposed for 5 years, but incomplete reporting of the results precluded establishing with certainty the dose level at which this effect occurred.

### 3. HEALTH EFFECTS

#### 3.1 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 benzidine. 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.

The toxicity and toxicokinetic information presented in this chapter focuses on studies that involved benzidine, either in its free base or dihydrochloride forms (all doses are expressed in terms of the free base). Data from various benzidine derivatives (including benzidine-based dyes) are occasionally mentioned for comparative purposes, or because such chemicals may serve as potential sources of benzidine exposure.

#### 3.2 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-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "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

### 3. HEALTH EFFECTS

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 (LOAELs) 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 benzidine are indicated in Tables 3-1 and 3-2 and Figures 3-1 and 3-2. Because cancer effects could occur at lower exposure levels, Figures 3-1 and 3-2 also show 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 and the MRLs.

#### **3.2.1 Inhalation Exposure**

Benzidine is a solid with a low vapor pressure (see Table 4-1) at normal ambient temperatures; therefore, inhalation of the vapor would not commonly be expected in the general environment. However, in the past, many occupational exposures involved inhalation of airborne dust or vapors from benzidine. Although exposure by the dermal route may also have been significant, most epidemiological studies will be discussed in the Inhalation Exposure section rather than in the Dermal Exposure section.

### 3. HEALTH EFFECTS

#### 3.2.1.1 Death

No studies were located regarding death in humans or animals after acute- or intermediate-duration inhalation exposure to benzidine.

Death from cancer following chronic-duration occupational exposure of workers is discussed in Section 3.2.1.8.

Of 48 rats exposed to benzidine dust in air (10–20 mg/m<sup>3</sup>), 20 died within 13 months (Zabezhinskii 1970). However, the significance of this is unclear because neither survival in the control population nor information on the causes of death was reported.

#### 3.2.1.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, or ocular effects in humans or animals after inhalation exposure to benzidine.

#### 3.2.1.3 Immunological and Lymphoreticular Effects

Total natural killer cell activity against target cells from a human cancer cell line was found to be normal in 63 Japanese workers occupationally exposed to benzidine and  $\beta$ -naphthylamine when compared to unexposed workers from the same factory (Tanigawa et al. 1990). However, the number of circulating natural killer cells actually mediating cytotoxic activity was increased in exposed workers relative to controls, indicating a decrease in cytotoxic activity per natural killer cell. Mechanisms to explain this apparent effect of benzidine (and/or  $\beta$ -naphthylamine) on natural killer cell activity, and particularly its persistence for nearly 20 years, are currently unknown. It is important to note that these data do not prove that benzidine exposure actually impairs immune system function since the observed differences between exposed workers and controls may have been specific to  $\beta$ -naphthylamine and not benzidine.

Furthermore, no attempt was made to statistically account for smoking, although there was a similar number of smokers in both groups. In another study, the blood of 27 high-exposure and 25 low-exposure male dye workers who had been exposed to  $\beta$ -naphthylamine and benzidine prior to 1972 was analyzed for T lymphocyte subpopulations, Leu11a-(CD-16) positive lymphocytes, and total lymphocytes (Araki et al. 1993). These values were compared to a control group of 33 workers at the same plant that

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had no known exposure to aromatic amines. The total and relative numbers of CD4+ and CD3+ lymphocytes in the high-exposure group were significantly lower than those in the control group, but there were no significant differences regarding CD8+, CD16+, and total number and percentage of lymphocytes. There were no significant differences between the 25 low-exposure workers and the 33 subjects in the control group with respect to the parameters examined. Some people in the group examined by Araki et al. (1993) may have been also subjects in the Tanigawa et al. (1990) study; therefore, the observations may not be independent. Sung et al. (1995) and Tanigawa et al. (1996) reported on male dye workers also exposed before 1972. High-exposure workers exhibited a reduction in the relative number of total CD4+ T lymphocytes and concurrent reductions in the absolute and relative numbers of subpopulation CD4+CD45RA+ (suppressor-inducer) T lymphocytes; the authors suggested that this T lymphocyte subpopulation may be the major target of effects related to aromatic amine exposure (Sung et al. 1995). Tanigawa et al. (1996) found significantly increased numbers of natural killer cell subpopulation CD57+CD16- in high-exposure workers, and indicated that this increase might be compensation for observed decreases in the number of CD4+ lymphocytes. It is important to note that none of the study subjects exhibited signs of infection at the time of the study; the authors also stated that the subjects were not taking any drugs (at the time of the study) which might have affected immunological analysis (Sung et al. 1995; Tanigawa et al. 1996).

No studies were located regarding immunological effects in animals after inhalation exposure to benzidine.

No studies were located regarding the following effects in humans or animals after inhalation exposure to benzidine:

#### **3.2.1.4 Neurological Effects**

#### **3.2.1.5 Reproductive Effects**

#### **3.2.1.6 Developmental Effects**

#### **3.2.1.7 Cancer**

A number of epidemiological studies of occupationally exposed workers have demonstrated that benzidine exposure is associated with an elevated risk of developing urinary bladder cancer (Bulbulyan et al. 1995; Goldwater et al. 1965; Meigs et al. 1986; Montanaro et al. 1997; Naito et al. 1995; Shinka et al. 1991; Vigliani and Barsotti 1962; Zavon et al. 1973). Both inhalation and dermal routes of exposure are

## 3. HEALTH EFFECTS

likely involved in most of these studies; therefore, none of the available data are adequate to permit the establishment of accurate inhalation dose-response relationships. The power of these studies to find an association between exposure to benzidine and increased incidence of bladder cancer varies greatly from study to study, largely due to the different sample sizes and to the difficulty of determining the extent to which workers were exposed to benzidine and/or other carcinogens. Acknowledging that while some studies may have evaluated a less than optimal number of individuals, the number of studies that found a positive association between bladder cancer and exposure to benzidine leave little uncertainty regarding the true nature of such an association. Most studies located were cohort studies, but a few case control studies were also available. In general, it is likely that the level of exposure in the case control studies is, on average, much lower than in the cohort studies, which tend to focus on workers with highest benzidine exposures. It should also be mentioned that in case control studies, information is collected on past events which may be inaccurately recorded or not available, and information supplied by an informant may be consciously or unconsciously biased. Thus, because of over exposures and the potential for misclassification, case control studies are less useful than cohort studies in making causal statements.

Among 83 Italian dye-stuff workers exposed to benzidine between 1931 and 1960, 20 were diagnosed with bladder tumors (Vigliani and Barsotti 1962). Measured airborne concentrations of benzidine ranged from 0 to 2.0  $\mu\text{g}/\text{m}^3$  (mean=0.3  $\mu\text{g}/\text{m}^3$ ), and urinary concentrations ranged from 6 to 26  $\mu\text{g}/\text{L}$ . Similar findings were reported for a coal tar dye factory, where 17 of 76 workers exposed to benzidine between 1912 and 1962 developed bladder cancer (Goldwater et al. 1965). An English case control study of 1,030 bladder patients showed an elevated risk for developing bladder cancer among textile and dye workers with the potential for benzidine exposure (Anthony and Thomas 1970). Working in the textile industry or as a dye worker was associated with 2-fold and 7-fold increases in bladder cancer risk, respectively. Two control groups were used in this study, patients with cancer at other sites and surgical patients who did not have cancer. Among 105 male workers at a Connecticut benzidine manufacturing facility who were estimated to have 2 or more years of exposure, 6 bladder tumors were observed (standardized incidence ratio=1,303) when only 0.46 were expected (Meigs et al. 1986). No significant increases were found for other anatomical sites. In a case report described by Tsuchiya et al. (1975), there were 65 workers who developed bladder tumors among an estimated 542 workers (11.3%) involved in the production of benzidine, compared to 11 of 761 (1.4%) involved with its use.

A cohort of 25 males occupationally exposed to benzidine during its manufacture was followed for 13 years (Zavon et al. 1973). Although the common chemical to which all were exposed was benzidine, exposure to other suspect chemicals could not be entirely discounted. Airborne benzidine concentrations

## 3. HEALTH EFFECTS

at various locations within the plant varied from  $<0.005$  to a maximum of  $17.6 \text{ mg/m}^3$  at a location where the workers shoveled benzidine into drums; the approximate mean urinary concentration reached  $0.04 \text{ } \mu\text{g/L}$  by the end of the workshift. Thirteen men (52%) developed transitional cell bladder carcinoma after a mean exposure of 13.6 years and an average latency (time from first exposure) of 16.6 years. The mean duration of exposure for those who did not develop tumors was 8.9 years. No cases of tumors were observed in any man who spent less than six years in the production of benzidine. Four renal tumors were also observed. Of four men who had died, only one died as a result of urinary tract malignancy, and he had refused all therapy after the initial treatment. The other three died from causes unrelated to urinary tract pathology. Zavon et al. (1973) stated that (at the time of the study) the mean survival time for all 13 tumor patients was 10 years, and 9.7 years for the 11 patients with conditions diagnosed as cancer. Although dermal exposure was also likely, it appears that inhalation was the major route of exposure.

Age at first exposure to benzidine has been correlated with bladder cancer risk in several epidemiological studies. Shinka et al. (1991) observed that 105 of 874 Japanese workers who were engaged in the manufacture and handling of benzidine (but not  $\beta$ -naphthylamine) developed urothelial (primarily bladder) tumors. The approximate mean latency period was 24 years (consistent with peak tumor incidence occurring 25 years after peak benzidine production), and varied inversely with the worker's age at first exposure. In a more recent study of 363 dye workers, Shinka et al. (1995) found urothelial tumors in 58 of the workers. The risk factors significantly related to tumor occurrence were benzidine as a dye intermediate (odds ratio [OR], 8.302,  $P<0.05$ ), manufacturing work (OR, 4.631,  $P<0.01$ ), and duration of exposure (OR, 1.018,  $P<0.05$ ). Relative risk of bladder cancer mortality in a cohort of 664 Italian workers exposed to various aromatic amines, including benzidine, was also found to vary inversely both with age at first exposure and time since last exposure (Piolatto et al. 1991).

In a retrospective cohort study of 1,972 Chinese workers exposed to benzidine between 1972 and 1977, the standardized incidence and mortality ratios for bladder cancer were 25 and 17.5, respectively (Bi et al. 1992). Further, bladder cancer was positively correlated with both estimated exposure level and exposure duration. The authors noted that occupational risk of benzidine-associated bladder cancer appeared to be comparable for Chinese and western workers, despite a significantly lower prevalence in Chinese workers of the slow-acetylator phenotype associated with increased bladder cancer risk (see Sections 3.5.2 and 3.10).

An industrial hygiene study identified benzidine and/or its metabolite N-acetylbenzidine in the urine of 8 of 38 workers exposed to benzidine-based dyes (NIOSH 1980c). Several of these workers were in the



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textile dyeing industry rather than in dye manufacture, and thus were primarily exposed to the dyes, not free benzidine (contaminant levels of benzidine in the dyes were <1–20 ppm). This suggests that individuals may be indirectly exposed to benzidine via the reduction of benzidine-based dyes to benzidine, and that occupational exposure to such dyes may present an excess risk for cancer of the bladder or other sites. Somewhat elevated relative risks for bladder cancer have been noted in textile dyers (Anthony and Thomas 1970). However, no similar excess risk for workers in the Chinese textile printing and dyeing industry was found, despite a demonstrated occupational link between benzidine or benzidine-based dye manufacture and worker bladder cancer (Xue-Yun et al. 1990). One possible explanation for this latter result is that there was little actual exposure to the benzidine-based dyes (or to contaminating benzidine); however, no exposure data were reported. The data of Xue-Yun et al. (1990) also provide support that there is no relationship between exposure to benzidine-based dyes and increased bladder cancer risk.

Benzidine-exposed workers who also smoked cigarettes were reported to have nearly a 31-fold higher risk of developing bladder cancer compared to an 11-fold risk of developing bladder cancer among nonsmoking coworkers (Bi et al. 1992). This synergistic effect of smoking on benzidine-associated bladder cancer risk was also observed in a retrospective cohort study of 2,525 Chinese workers exposed to benzidine for at least 1 year (Wu 1988). When compared with nonsmoking controls (nonexposed), the relative risk for bladder cancer of smoking controls was 6.2, compared to a risk of 63.4 for nonsmoking exposed workers, and a risk of 152.3 for smoking exposed workers. The potential role of increased benzidine ingestion due to the increased hand-to-mouth activity associated with smoking was not addressed. For all males exposed to benzidine, the standardized incidence ratio was calculated at 2,610, in remarkably close agreement with the standardized incidence ratio of 2,500 from the previously discussed findings of Bi et al. (1992).

Although there have been some studies to the contrary (Meigs et al. 1986; Naito et al. 1995), there is some evidence suggesting that exposure to benzidine may elevate the risk of developing cancer at sites other than the bladder. However, the evidence supporting such an association is much weaker than that for bladder cancer. Contributing to the weakness of the evidence is the fact that the number of cases is usually small, such that the biological significance of any statistical analysis is questionable and also, there seems to be little consistency in the findings regarding tumor sites. Based on what is known about the mechanism of carcinogenicity of benzidine (see Sections 3.4.3 and 3.5.2), findings of carcinogenicity in the liver and kidney, as some studies have reported, are not totally unexpected.

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In the previously mentioned study by Wu (1988), in addition to the principal findings related to bladder cancer, a slight increase in the incidence of lung and stomach cancers was noted in workers exposed to benzidine, but quantitative data were not provided. In a study of 639 white males in Ohio exposed to benzidine and/or  $\beta$ -naphthylamine during 1938 and 1939 and followed until 1965, Mancusco and El-Attar (1967) reported not only excess bladder cancer, but also six cases of pancreatic cancer. The calculated mortality rates per 100,000 due to pancreatic cancer were 39 for the exposed workers and 7.5 for the general white male population of Ohio. Though of questionable significance, one case of kidney cancer was also observed.

A large cohort of 3,322 Japanese workers exposed to benzidine and/or  $\beta$ -naphthylamine between 1950 and 1978 was studied by Morinaga et al. (1982). The observed period of the study cohort ranged from 0.1 to 22 years (mean=8.2 years) and that of a control cohort from 0.3 to 13.5 years (mean=5.3 years). The control cohort consisted of 177 male unexposed patients with bladder cancer. Among employees exposed to benzidine and/or  $\beta$ -naphthylamine, 244 workers were found to have suffered from and consequently died of cancer of the genitourinary organs (first primary cancer). Of these, 11 (4.5%) developed second primary cancers of the liver, gallbladder, bile duct, large intestine, or lung. For the liver, gallbladder, and bile duct cases, the difference between the study cohort and control group was statistically significant ( $p < 0.05$ ). The first primary cancers in these 11 cases were all transitional cell carcinomas of the urinary tract. The second primary cancers were all adenocarcinomas except for a cancer of the maxillary sinus. Similar findings were reported in another Japanese study comparing 117 bladder cancer patients exposed to benzidine and/or  $\beta$ -naphthylamine with 117 nonexposed control bladder cancer patients (Okubo et al. 1985). Multiple primary cancers were observed in eight of the exposed bladder cancer cases compared to only one of the control cases (three adenocarcinomas of the colon and/or rectum, three adenocarcinomas of the lung, one adenocarcinoma of biliary tract, and one hepatoma). Interestingly, the study also showed that benzidine-exposed workers developing bladder cancer had shorter survival times than persons without benzidine exposure who developed bladder cancer. This could indicate either that there are more multiple primaries in benzidine workers (bladder cancer among benzidine workers is more deadly), or that benzidine workers are diagnosed later with bladder cancer, which is unlikely given that they are under surveillance. A case of kidney, bladder, and liver cancer was recently reported in a Japanese dye worker at autopsy (Morikawa et al. 1997). Analyses of the tumors led to a diagnosis of triple primary tumors. The worker had been exposed by inhalation for eighteen years to benzidine, but not to  $\beta$ -naphthylamine or other carcinogens.

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A cohort of workers exposed to azodye-related compounds, including benzidine, was examined as part of a larger retrospective study of 2,642 workers employed at a New Jersey dye and resin manufacturing plant from 1952 to 1985 (Delzell et al. 1989). The mortality experience of the workers was related to former employment at the Cincinnati Chemical Works, which had produced or used benzidine and  $\beta$ -naphthylamine. The 2,553 workers who had never worked at the Cincinnati Chemical Works and therefore, had little potential for benzidine exposure, had fewer than expected deaths from all causes combined and equal numbers of observed and expected cancer deaths. The 89 former Cincinnati Chemical Works workers had an excess of cancer (17/8.6,  $P=0.02$ ), which was due to excess mortality from bladder (3/0.25,  $P=0.004$ ), kidney (2/0.21,  $P=0.04$ ), and central nervous system (2/0.22,  $P=0.04$ ) cancers. Further evidence suggesting the potential for additional target organ sites for benzidine carcinogenicity was found in the previously discussed study of 664 Italian dyestuff workers (Piolatto et al. 1991). In that study, elevated ratios of observed-to-expected deaths were noted for cancer of the bladder (49/1.6), oral cavity (6/2.2), esophagus (4/1.7), and larynx (9/2.4).

The mortality of a cohort of 1,244 workers (870 men, 374 women), exposed to benzidine-based azo dyes (as well as other chemicals used in the tanning process) during employment at an Italian chrome tannery between 1955 and 1988, was studied until 1994 (Montanaro et al. 1997). While all-cause mortality was similar to that of the general population, mortality for all cancers was 12% higher than expected. Observed (10) versus expected (4.13) deaths due to bladder cancer yielded a standardized mortality ratio (SMR) of 242. An excess of colorectal cancers was also reported (17 observed, 9.4 expected,  $SMR=180$ ). An earlier study by Siemiatycki et al. (1994) found no excess bladder or other cancer risk among leather workers; it should be noted, however, that the authors did not assess exposure to specific amines, but only to aromatic amines as a class.

Cancer incidence and mortality were evaluated among aniline dye workers employed in a Moscow facility as of January 1975, and followed until December 1989 (Bulbulyan et al. 1995). In a group of men (514) and women (287) who had ever been exposed to benzidine or  $\beta$ -naphthylamine during a combined total of 9,048 person-years of employment, there were 115 observed cases of all cancers versus 62.57 expected cases. The ratios of observed/expected cases of bladder cancer were 19/1.76 (standardized incidence ratio 1,082) in men and 5/0.24 (standardized incidence ratio 2,097) in women. The risk for bladder cancer did not increase with duration of employment for workers ever exposed to benzidine, but it did for workers ever exposed to  $\beta$ -naphthylamine. Excess risks were found in men for esophageal and lung cancer. Excess cancer rates, including bladder cancer, were also found in groups of employees considered to have been exposed only to "other" chemicals.

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In a case control study, Wilkins and Sinks (1990) analyzed parental occupational histories for 110 cases of childhood malignant neoplasm of the brain, and for 193 matched controls. A cluster methodology was used to group jobs with similar exposures to certain chemicals. In jobs categorized as having moderate to high exposure to aromatic amines, paternal employment during preconception, prenatal, or postnatal periods was associated with the following elevated odds ratios (with 95% confidence intervals): 2.4 (0.7–7.8), 1.8 (0.5–6.2), and 2.7 (1.1–6.8), respectively. When paternal exposure to benzidine during the postnatal period was inferred to be from moderate to high, there was an associated elevated odds ratio of 4.3 (1.3–13.8). In both the prenatal and preconception periods, the frequency of moderate-to-heavy occupational exposure among fathers was low and no odds ratios were calculated for benzidine. Few maternal exposure associations were identified, probably due to the small number of employed mothers in the study. The value of this study is weakened by ill-defined exposure routes (inhalation is presumed to be the primary route for benzidine exposure), as well as the lack of sufficient validation of the job exposure matrix used in estimating exposure levels. The results and conclusions of this study are largely speculative, and considerably more evidence will be required to draw any conclusions with confidence.

Two studies that did not find significant increased incidences of cancers other than bladder cancer are Meigs et al. (1986) and Naito et al. (1995). In a study of workers at a Connecticut benzidine manufacturing facility, Meigs et al. (1986) did not find significantly elevated risks for cancers at sites other than the bladder in men or at any anatomic site in women, and no pattern of increasing risk with increasing benzidine exposure for sites other than the bladder. The authors did find, however, nonstatistically significant increases in the incidence of prostate cancer, stomach cancer, and lung cancer; in the latter case, 8 of 11 subjects had a history of heavy smoking, and no information was available for the other three. In the Naito et al. (1995) study, a cohort of 442 workers (437 men, 5 women) exposed to one or more substances including benzidine at a dyestuff factory in Japan during employment between the years of 1935 and 1988, was followed until December 1992 (average observation period, 39.4 years). A significant increased risk for bladder cancer was found among workers engaged in the manufacture (SMR=63.6) and use (SMR=27) of benzidine, but the increased risk for cancer mortality for other organs was not significant. It was also found in this study that the risk of bladder cancer increased with duration of exposure to benzidine.

In the sole reported animal inhalation study, outbred albino rats of both sexes were exposed to 10–20 mg/m<sup>3</sup> of benzidine dust in air 4 hours/day, 5 days/week for 20 months (Zabehzhinskii 1970). Of the 28 experimental rats that survived beyond 13 months, 5 were found to have myeloid leukemia, 2 had fibroadenoma of the mammary gland, 1 had a squamous carcinoma of the male mammary gland, 1 had a

### 3. HEALTH EFFECTS

hepatoma, and 1 had a mammary adenocarcinoma. Among the 21 surviving control rats, 2 had mammary gland adenomas. No urinary bladder tumors were reported in either group. The earliest carcinogenic effect (myeloid leukemia) was noted in an exposed animal after 13 months of the study. This CEL value for cancer in rats is recorded in Table 3-1 and plotted in Figure 3-1. Several study weaknesses should be noted. Only 28 of 48 (58%) experimental animals survived to 13 months. It is not clear whether this is an indication that the Maximum Tolerated Dose (MTD) was exceeded, because survival in the control group may have also been low (21 control rats survived until this time). However, the initial number of control animals used was not reported, and possible reasons for poor survival in either the experimental or control groups were not discussed. Further, the purity of the benzidine used was not reported, and no mention was made of particle size distribution, or possible dermal and oral absorption from dust accumulation on the skin and grooming, respectively. Therefore, this study can only be considered inconclusive evidence that inhalation of benzidine can produce cancer in rats.

EPA has derived an inhalation cancer potency factor of  $2.3 \times 10^2 \text{ (mg/kg/day)}^{-1}$  using the linearized multistage model (EPA 1999a). This factor was derived from data on bladder tumor incidence in humans occupationally exposed to benzidine as described by Zavon et al. (1973). The corresponding inhalation unit risk is  $6.7 \times 10^{-2}$  per  $\mu\text{g}/\text{m}^3$ . The air concentrations corresponding to excess cancer risk levels of  $1 \times 10^{-4}$ ,  $1 \times 10^{-5}$ ,  $1 \times 10^{-6}$ , and  $1 \times 10^{-7}$  are  $1.5 \times 10^{-6}$ ,  $1.5 \times 10^{-7}$ ,  $1.5 \times 10^{-8}$ , and  $1.5 \times 10^{-9}$   $\text{mg}/\text{m}^3$ , respectively, as indicated in Figure 3-1.

#### 3.2.2 Oral Exposure

Few data have been reported on the toxicity (other than carcinogenicity or genotoxicity) of benzidine after oral exposure. However, significant oral exposure to benzidine is unlikely, particularly for populations not located near waste sites or dye production facilities. Ingestion of benzidine-based dyes may be a more likely occupational exposure scenario than ingestion of benzidine. It has been shown that ingestion of benzidine-based dyes can cause effects in animals similar to effects caused by ingestion of benzidine, and that these dyes can be converted metabolically and environmentally to benzidine (Gregory 1984). See also Chapter 6.

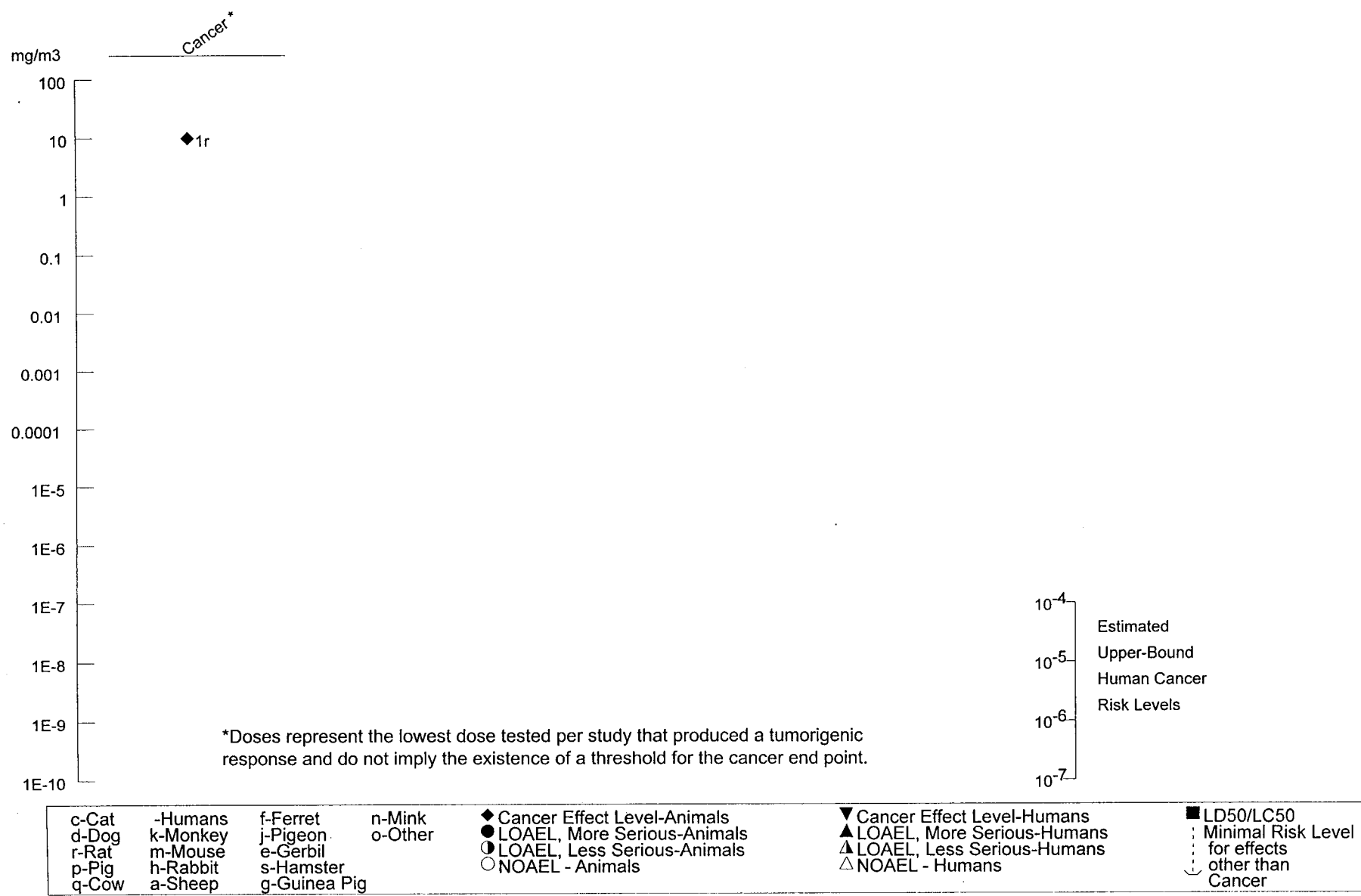
Table 3-1. Levels of Significant Exposure to Benzidine - Inhalation

Key to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL mg/m <sup>3</sup>	LOAEL		Reference
					Less Serious mg/m <sup>3</sup>	Serious mg/m <sup>3</sup>	
<b>CHRONIC EXPOSURE</b>							
<b>Cancer</b>							
1	Rat (albino)	20 mo 4hr/d 5d/wk				10	(CEL: leukemia; mammary fibroadenoma, squamous carcinoma, adenocarcinoma) Zabezhinskii 1970

\*The number corresponds to entries in Figure 3-1.

CEL = cancer effect level; d = day(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; m<sup>3</sup> = cubic meter(s); mg = milligram(s); mo = month(s); NOAEL = no-observed-adverse-effect level; wk = week(s)

Figure 3-1. Levels of Significant Exposure to Benzidine - Inhalation  
Chronic (≤365 days)



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**3.2.2.1 Death**

No studies were located regarding death in humans after oral exposure to benzidine. The reported oral LD<sub>50</sub> is 214 mg/kg body weight for mice and 309 mg/kg body weight for rats (DOT 1972). Oral doses approximating 200 mg/kg are also estimated to have caused the deaths of rabbits and dogs (Adler 1908).

All reliable LOAEL values for death in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

**3.2.2.2 Systemic Effects**

No studies were located regarding respiratory, gastrointestinal, musculoskeletal, dermal, or ocular effects in humans or animals after oral exposure to benzidine. The systemic effects observed after oral exposure are discussed below. The highest NOAEL values and all LOAEL values from each reliable study for observed systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

**Cardiovascular Effects.** No studies were located regarding cardiovascular effects in humans after oral exposure to benzidine.

One study reported that benzidine administered orally to 6 rabbits (50 or 100 mg 1 day/week, or approximately 13 or 26 mg/kg/day), and 2 dogs (100 mg 1 day/week, or approximately 7.9 mg/kg/day) for 20–128 days induced myocardial atrophy and interstitial myocarditis (Oida 1958a, 1958b). Five rabbits died during the study and the cause of death was not reported; also, no controls were used. This study is not considered reliable for the assessment of cardiovascular effects and, therefore, is not included in Table 3-2. No other studies were located regarding cardiovascular effects in animals after oral exposure to benzidine.

**Hematological Effects.** No studies were located regarding hematological effects in humans after oral exposure to benzidine.

Mice were provided water containing 30–400 ppm benzidine dihydrochloride (equivalent to 2.5–26.2 mg/kg/day of benzidine base) for 40, 60, and 80 weeks (Nelson et al. 1982). Hemosiderin



Table 3-2. Levels of Significant Exposure to Benzidine - Oral

Key to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
<b>ACUTE EXPOSURE</b>							
<b>Death</b>							
1	Rat (NS)	NS (G)				309 (LD <sub>50</sub> )	DOT 1972
2	Mouse (NS)	NS (G)				214 (LD <sub>50</sub> )	DOT 1972
<b>INTERMEDIATE EXPOSURE</b>							
<b>Systemic</b>							
3	Rat (Sprague-Dawley)	6 wk (W)	Hepatic		5.3 (bile duct hyperplasia)		DePass and Morris 1981
			Renal		5.3 (proteinaceous casts in tubules)		
			Bd Wt			5.3 (31% decrease in weight gain)	
4	Mouse (BALB/c)	6 wk (W)	Hepatic	32			DePass and Morris 1982
			Bd Wt	32			
<b>Cancer</b>							
5	Rat (Sprague-Dawley)	9 mo 1x/3d (GO)				3.4 F (CEL: mammary carcinoma)	Griswold et al. 1968

Table 3-2. Levels of Significant Exposure to Benzidine - Oral (continued)

Key to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
<b>CHRONIC EXPOSURE</b>							
<b>Systemic</b>							
6	Mouse (BALB/c) M (C57BL/6) F	Lifetime (W)	Hemato	7.2	10.7 (hemosiderosis of the spleen)		Littlefield et al. 1983, 1984
			Hepatic Bd Wt	3.7 F	5.6 F (bile duct hyperplasia)	1.8 F (28% reduction in body weight gain)	
7	Mouse (BALB/c) M (C57BL/6) F	40-80 wk (W)	Bd Wt		2.5 (decreased body weight gain greater than 10%)		Schieferstein 1982
8	Dog (NS)	5 yr 6d/wk (C)	Renal		17.6 (recurrent cystitis)		Spitz et al. 1950
<b>Neurological</b>							
9	Mouse (BALB/c) M (C57BL/6) F	Lifetime (W)				1.8 F (spongiform leukoencephalopathy)	Morgan et al. 1981; Littlefield et al. 1983
<b>Reproductive</b>							
10	Mouse (BALB/c) M (C57BL/6) F	lifetime				7.2 F (atrophy of the uterus)	Littlefield et al. 1983
<b>Cancer</b>							
11	Mouse (BALB/c) M (C57BL/6) F	Lifetime (W)				1.8 F (CEL: Harderian gland adenoma)	Littlefield et al. 1983
12	Mouse (BALB/c) M (C57BL/6) F	Lifetime (W)				1.8 F (CEL: liver carcinomas)	Littlefield et al. 1984

Table 3-2. Levels of Significant Exposure to Benzidine - Oral (continued)

Key to <sup>a</sup> figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL		Reference	
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)		Serious (mg/kg/day)
13	Mouse (BALB/c) M (C57B/6) F	40-80 wk (W)				2.5 F (CEL: liver adenomas, carcinomas)	Nelson et al. 1982
14	Hamster (NS)	Lifetime (F)				61 (CEL: bile duct and liver adenomas, carcinomas)	Saffiotti et al. 1967
15	Dog (NS)	5 yr 6d/wk (C)				24.2 (CEL: bladder carcinomas)	Spitz et al. 1950

<sup>a</sup>The number corresponds to entries in Figure 3-2.

Bd Wt = body weight; (C) = capsule; CEL = cancer effect level; d = day(s); (F) = feed; F = female; (G) = gavage; (GO) = gavage in oil; Hemato = hematological; kg = kilogram(s); LD<sub>50</sub> = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mg = milligram(s); mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; (W) = water; wk = week(s); x = times; yr = year(s)

Figure 3-2. Levels of Significant Exposure to Benzidine - Oral  
Acute ( $\leq 14$  days)

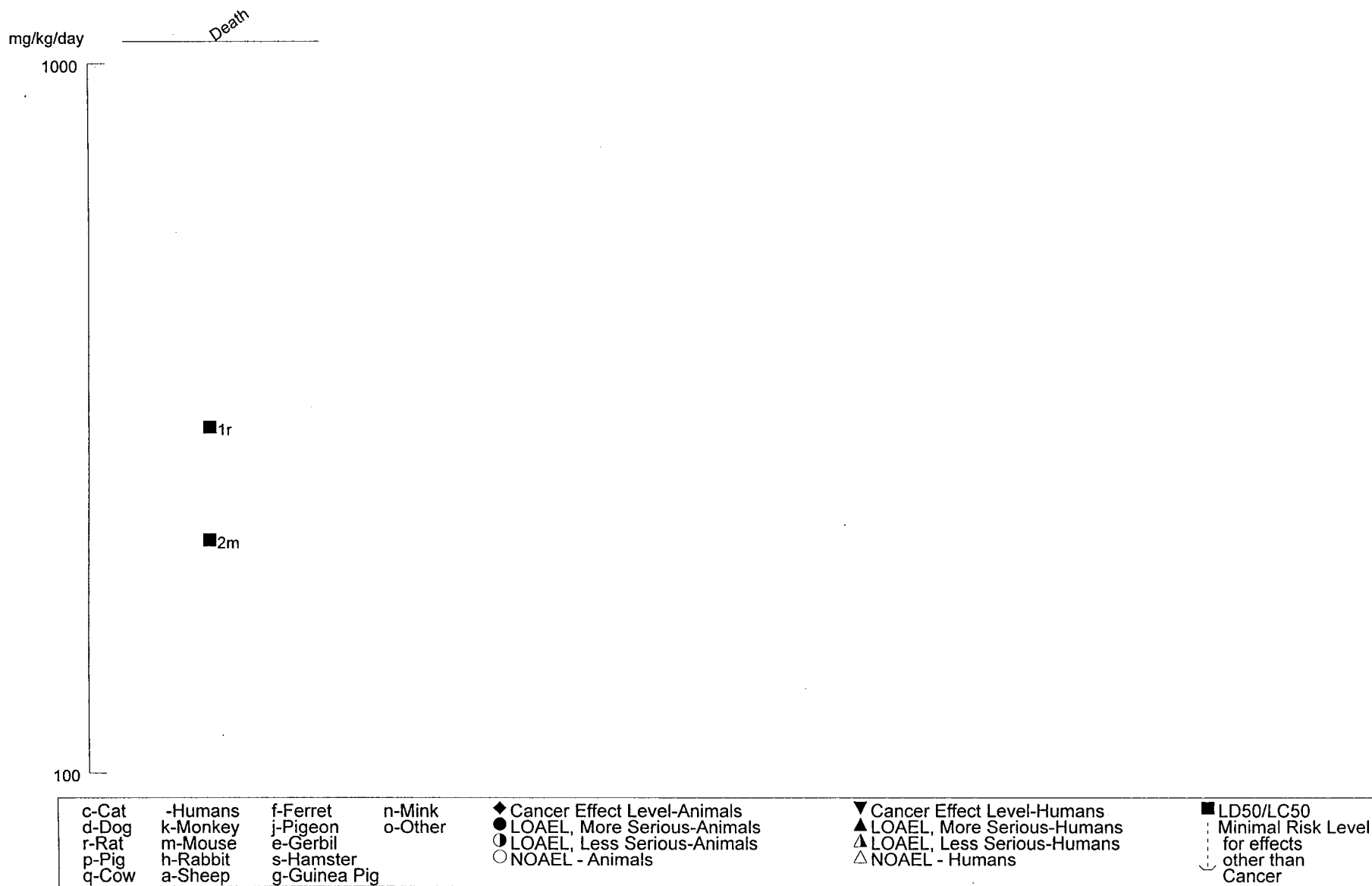
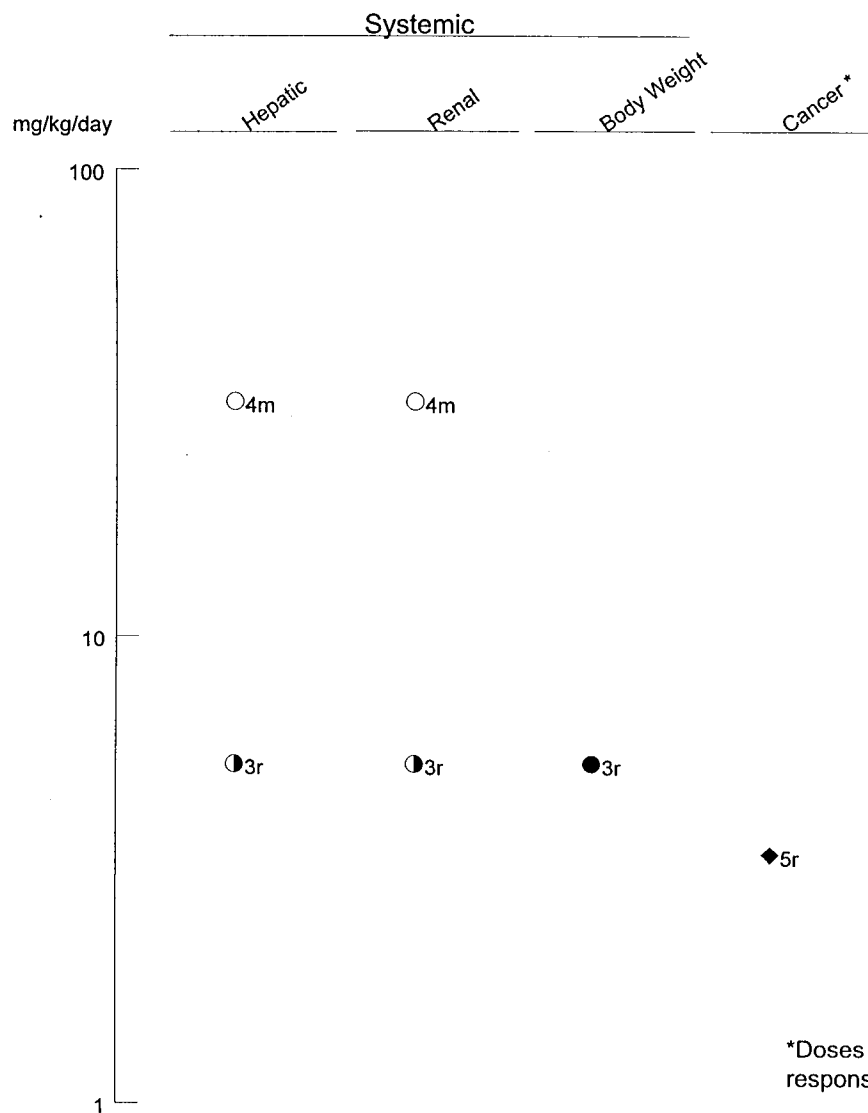


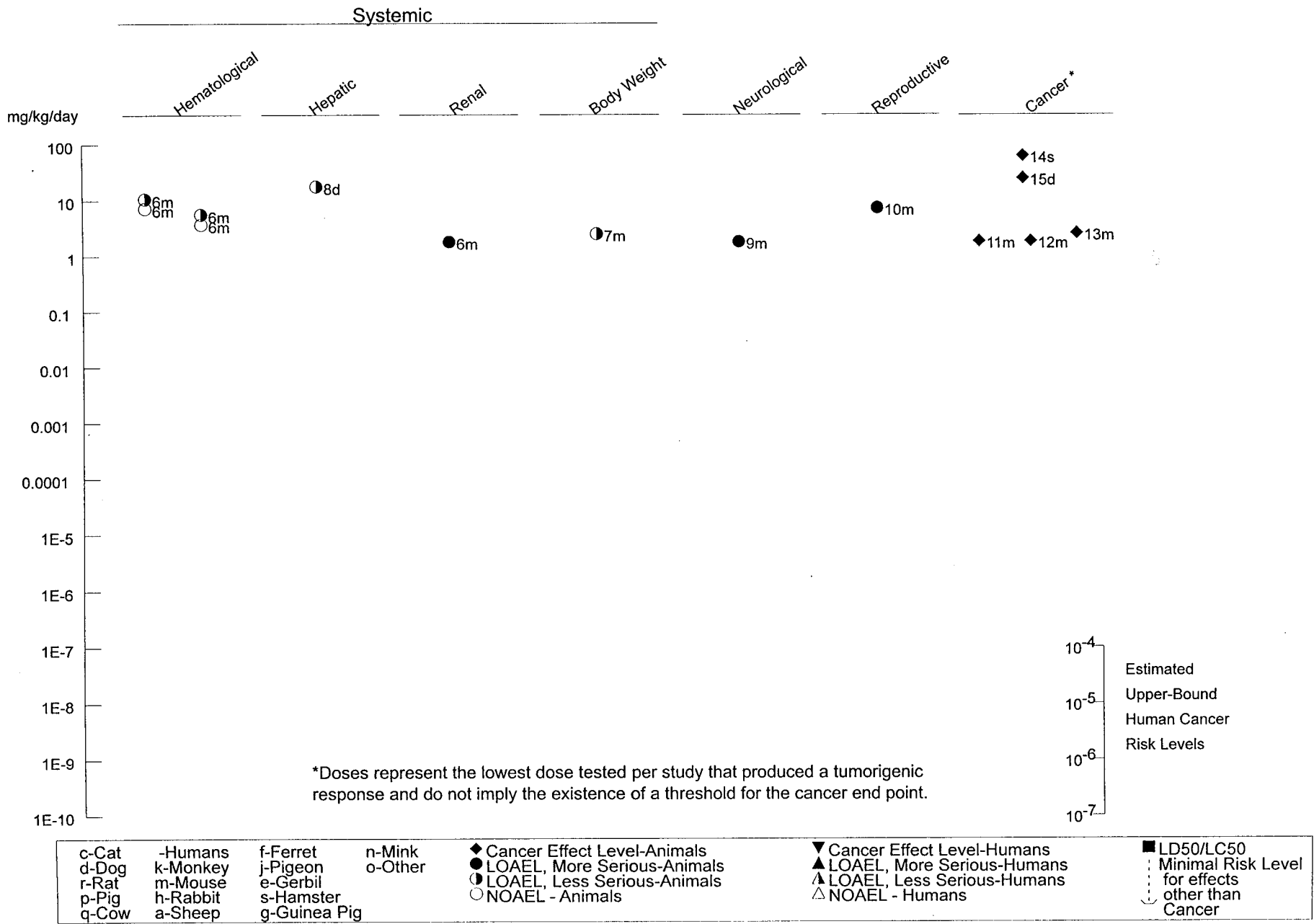
Figure 3-2. Levels of Significant Exposure to Benzidine - Oral (continued)  
Intermediate (15-364 days)



\*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	⋮ Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◐ LOAEL, Less Serious-Animals	△ LOAEL, Less Serious-Humans	⋮ for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

Figure 3-2. Levels of Significant Exposure to Benzidine - Oral (continued)  
Chronic (≥365 days)



## 3. HEALTH EFFECTS

pigment in the spleen was found in significant amounts for both sexes of the two crosses examined (F<sub>1</sub> hybrid of C57BL and BALB/C3H strains and subsequent monohybrid cross) at the 40- and 60-week terminations. The increase in the level of pigment was also significant at the 80-week termination in the males. Erythropoiesis in the spleen was significantly increased in females (but not males) for both strains at the 60- and 80-week terminations. The doses at which these effects were first observed were not reported. In another mouse study, macrophages containing hemosiderin pigment were observed in the spleen after lifetime exposures to benzidine at doses of 10.7–12.0 mg/kg/day, but not at 0–7.2 mg/kg/day (Littlefield et al. 1983, 1984). Though typical hematological examinations were not conducted in these studies, the results suggest that benzidine may be capable of damaging erythrocytes, with a resulting deposit of hemosiderin in the spleen.

**Hepatic Effects.** No studies were located regarding hepatic effects in humans after oral exposure to benzidine.

Cirrhosis of the liver was found in rabbits (perhaps also in dogs, but this was not clearly indicated) orally exposed to approximately 13 or 26 mg/kg/day of benzidine, 1 day/week over a period of 20–128 days (Oida 1958a, 1958b). However, five of the six rabbits died during the study and the cause of death was not reported. Also, no controls were used. This study is not considered reliable for the assessment of hepatic effects and, therefore, is not included in Table 3-2. Cirrhosis of the liver was observed in another rabbit study 8 months following treatment with benzidine (0.5–7.0 grams total dose over periods of up to 3.5 years) (Bonser 1959). The cirrhosis was frequently associated with changes in bile duct tissue. No further details were provided in this abstract; thus, this report is also not included in Table 3-2. Bile duct hyperplasia was observed in rats administered approximately 5.3 mg benzidine/kg/day in the drinking water for 6 weeks (De Pass and Morris 1981). Bile duct hyperplasia was also observed in a lifetime mouse study (Littlefield et al. 1983) at benzidine doses of 5.6–12.0 mg/kg/day, but not at lower doses of 0–3.7 mg/kg/day. The effect was more prevalent in females (21% at 10.7 mg/kg/day) than in males (8% at 12 mg/kg/day). The authors hypothesized about a possible hormone-mediated component of the response. No morphological alterations were observed in the livers from mice administered approximately 32 mg benzidine/kg/day in the drinking water for 6 weeks (DePass and Morris 1982).

**Renal Effects.** No studies were located regarding renal effects in humans after oral exposure to benzidine.

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Nephrosis, nephritis, hematuria, and proteinuria were reported in rabbits orally exposed to approximately 13 or 26 mg/kg/day of benzidine, 1 day/week over a period of 20–128 days (Oida 1958a, 1958b). However, five of the six rabbits died during the study and the cause of death was not reported. Also, no controls were used. This study is not considered reliable for the assessment of renal effects and, therefore, is not included in Table 3-2. Proteinaceous casts were observed in the renal tubules from rats dosed with approximately 5.3 mg benzidine/kg/day in the drinking water for 6 weeks (DePass and Morris 1981). Although not statistically significant ( $p < 0.10$ ), Nelson et al. (1982) observed proteinaceous casts in the kidneys of F<sub>1</sub> hybrid (C57BL x BALB/c) female mice exposed to benzidine for 60–80 weeks, but the particular doses (between 0 and 26.2 mg/kg/day) causing the effect were not identified. Hematuria and proteinuria were reported in two dogs within 2–5 weeks of being orally exposed to 7.9 mg/kg/day of benzidine, 1 day/week over a period of 20–128 days (Oida 1958a, 1958b). Even though no other visible signs of toxicity were noted, recurrent episodes of cystitis were observed in the bladders of seven dogs fed benzidine for 5 years (Spitz et al. 1950). The dogs were exposed by capsule to doses of approximately 17.6 mg/kg/day for the first 15 months, then 26.4 mg/kg/day for the remaining 45 months. Histologically, focal collections of lymphocytes were observed, often forming follicles. There was no notable reaction of the mucosa, and the lesions improved without therapy.

**Body Weight Effects.** No studies were located regarding body weight effects in humans after oral exposure to benzidine.

No significant treatment-related changes in body weight were reported in mice administered approximately 32 mg benzidine/kg/day in the drinking water for 6 weeks (DePass and Morris 1982). Schieferstein (1982) reported decreased body weight gain (greater than 10%) in mice exposed to benzidine at 2.5 mg/kg/day in the drinking water for 80 weeks. Also, Littlefield et al. (1983, 1984) reported that mice administered benzidine in the water for 2 years at dose levels of about 1.8 mg/kg/day gained approximately 28% less weight than controls.

#### 3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to benzidine.

Two animal studies have been reported that address the immunotoxicity of benzidine following oral exposure. In a study by Luster et al. (1992), an unspecified number of female B6C3F<sub>1</sub> mice were exposed by gavage for 5 days to three unspecified doses of benzidine, and were then subjected to a battery of



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immune response assays used to screen for potential immunotoxicity. Based on induced alterations in spleen/body weight ratio, natural killer cell activity, T-cell response to mitogen and mixed leukocyte antigens, and on a delayed hypersensitivity response, benzidine was classified as an immunotoxicant. Spleen cellularity, thymus/body weight ratio, number of B and T lymphocytes as quantified by cell surface markers, and number of splenic lymphocytes producing IgM antibody were not altered by benzidine exposure. Rao et al. (1971) reported hyperplasia of the lymphoid cells in the spleen and thymic cortex in mice exposed to benzidine dihydrochloride in the feed for 6 weeks; however, the lowest dose at which this effect occurred could not be determined from the study.

#### 3.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to benzidine.

Spongiform leukoencephalopathy (central nervous system damage resulting in vacuolization of white matter) was observed in mice after lifetime exposure to 20 ppm of benzidine dihydrochloride in drinking water (Littlefield et al. 1983; Morgan et al. 1981). These vacuoles may represent swollen glial cells and/or intramyelinic vacuoles between the larger axons. Using average weekly dose rate data provided by the authors in another publication concerning this study (Littlefield et al. 1984), 20 ppm was estimated to be equivalent to a benzidine dose of 1.8 mg/kg/day. The LOAEL value (1.8 mg/kg/day) from this study for neurological effects is recorded in Table 3-2 and plotted in Figure 3-2.

#### 3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to benzidine. The only information regarding reproductive effects in animals is that from a lifetime study in mice, which reported a 31% incidence of atrophy of the ovaries in animals given benzidine in the drinking water at a dose level of approximately 7.2 mg/kg/day (80 ppm) compared to 11% in control mice (Littlefield et al. 1983).

#### 3.2.2.6 Developmental Effects

In a 5-year retrospective study of residents living near a Superfund site contaminated with benzidine,  $\beta$ -naphthylamine, and benzene, no significant increase in incidence rate was detected for any of 37 most common birth defects (only 4 of which were observed), or in the rate for all combined birth defects

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(Budnick et al. 1984). However, the authors noted that the number of births was small, and that birth defects are often under-reported on birth certificates. The extent of actual exposure (if any) to benzidine was not determined.

No studies were located regarding developmental effects in animals after oral exposure to benzidine.

#### **3.2.2.7 Cancer**

A retrospective study of residents who lived, during the 1970s near a Superfund site, that was contaminated with benzidine,  $\beta$ -naphthylamine, and benzene detected an excess of bladder cancer, leukemia, and other lymphomas, as well as cancers at several other sites (salivary gland, larynx, bone and jaw, uterus and chorion, rectum, and breast) (Budnick et al. 1984). The principal routes of potential exposure were not identified, but are likely to have included oral exposure to contaminated water and soil. The specific contribution of environmental benzidine (if any), or of possible occupational exposure to benzidine or other carcinogens, could not be ascertained. A retrospective study of Japanese kimono painters who were exposed to benzidine-based dyes (Direct Black 38, Direct Red 28, Direct Red 17, and Direct Green 1) by licking their brushes and spatulas indicated an increased risk for bladder tumors (Yoshida 1971; Yoshida and Miyakawa 1973). Of 200 painters who worked for up to 50 years, 17 (8.5%) contracted bladder cancer. When compared with 148 controls having urinary disease, this represented a relative risk of 6.8 for these cohorts. The average age-adjusted death rate from bladder cancer for the general population in Japan during 1965 was reported to be less than 3 deaths per 100,000 persons. Although direct exposure to benzidine was not described, these data are consistent with the concept that benzidine-based dyes may be metabolically reduced to free benzidine, and thus cause the same cancer effects.

A number of animal studies indicate that oral exposure to benzidine can increase the incidence of a variety of tumors. In female rats given 3.4 mg/kg/day once every 3 days for 30 days, 5 out of 10 animals developed mammary carcinomas within 9 months, compared to 5 out of 132 in the controls (Griswold et al. 1968). In hamsters fed either 0.1% benzidine base (61 mg/kg/day) or 0.1% benzidine dihydrochloride (equivalent to 44 mg/kg/day benzidine base) in the lifetime diet, malignant and benign multiple cholangiomas, hepatomas, and/or liver carcinomas were reported in more than 50% of the surviving hamsters (Saffiotti et al. 1967). No bladder pathology was found in these animals.

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In a total of 13 rabbits fed a total dose of 0.5–7.0 grams of benzidine base in the diet for up to 3.5 years, one female developed a transitional cell carcinoma of the bladder after 2 years, while another female developed adenocarcinoma of the gallbladder with widespread metastasis at 6 years of age (Bonser 1959). The author concluded that two tumors occurring in organs of excretion in 13 rabbits is probably significant, and that it is possible that higher dosage levels would have caused more tumors. However, the incidence of tumors in control animals was not stated, making it difficult to assess the findings. No bladder tumors or other significant neoplasia were noted in rabbits intermittently given 13 or 26 mg/kg/day (50 or 100 mg weekly doses over 20–128 days; total doses of 0.1–1.4 grams) (Oida 1958a, 1958b). These reports were lacking in experimental details. The weekly dosing is difficult to evaluate and histopathological procedures were not adequately described, making the data of limited quantitative value.

Spitz et al. (1950) administered benzidine to 1 male and 6 female dogs by capsule for 60 months at a dose of 17.6 mg/kg/day (200 mg daily, 6 days/week for 15 months), followed by 26.4 mg/kg/day (300 mg daily, 6 days/week for 45 months). Dosing may have been suspended for brief periods because of recurring cystitis in dogs. One dog developed a urinary epidermoid carcinoma of the bladder at 19 months. It was later reported that three dogs (the longest survivors) of the Spitz et al. (1950) study developed papillomas and carcinomas of the bladder after 7, 8, and 10 years (Bonser 1959).

The most thorough animal study of oral carcinogenicity of benzidine was conducted at the National Center for Toxicological Research (NCTR) using two strains of male and female mice (a total of 3,456 animals) at five dose levels of benzidine dihydrochloride (Frith et al. 1979, 1980; Nelson et al. 1982; Schieferstein 1982). Doses (in water) were 30–400 ppm benzidine dihydrochloride (equivalent to benzidine doses of 2.5–26.2 mg/kg/day). The mice were sacrificed at 40, 60, and 80 weeks for pathological evaluations. Statistical analysis of the pathology data and the data pertaining to animal weights, water consumption, survival, and tumor incidences was performed. A series of publications resulted from this study. Hepatocellular alterations, hepatocellular adenomas, and hepatocellular carcinomas were all significantly elevated in one or more of the sex-strain combinations, even at the lowest dose level of 30 ppm (2.5 mg/kg/day). The primary target organ observed in the studies was the liver, where dose-response relationships could be observed. The data suggest that the lesions may first have appeared as foci of cellular alteration (acidophilic, basophilic, or vacuolated cellular foci), which may then have given rise to hepatocellular adenomas, which in turn progressed to hepatocellular carcinomas, the main cause of death among treated animals. These altered foci are thus considered preneoplastic lesions, an early stage in the progression from normal tissue to frank cancer.

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Hepatocellular carcinomas were also observed in mice given as little as 20 ppm of benzidine dihydrochloride in drinking water for about 33 months (Littlefield et al. 1983, 1984). From experimentally derived average dose rates in mice (Littlefield et al. 1984), this equates to a dose of 1.8 mg/kg/day which is presented in Table 3-2. At this same dose, there was also a substantial increase in the fraction of mice displaying Harderian gland adenomas (18–24%) when compared with controls (4–8%). The response generally leveled off at the mid and high doses. Other tumors that occurred at significantly greater rates than in controls were angioma of the uterus, lung tumors, and reticulum cell sarcomas. This study also provided information on latency and sex and strain differences. Female mice were significantly more susceptible to these adverse effects than male mice, as was the genetically more homogeneous F<sub>1</sub> stock (BALB/c males x C57BL/6 females) when compared with the more heterogeneous F<sub>2</sub>-monohybrid cross stock (F<sub>1</sub>x F<sub>1</sub>).

CEL values from each reliable study for cancer in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

EPA has derived an oral cancer potency factor of  $2.3 \times 10^2$  (mg/kg/day)<sup>-1</sup> using the linearized multistage model (EPA 1999a). This factor was calculated from inhalation exposure data reported by Zavon et al. (1973) regarding incidence of bladder tumors in humans occupationally exposed to benzidine. The lifetime average doses that would result in risks of  $1 \times 10^{-4}$ ,  $1 \times 10^{-5}$ ,  $1 \times 10^{-6}$ , and  $1 \times 10^{-7}$  are  $4.4 \times 10^{-7}$ ,  $4.4 \times 10^{-8}$ ,  $4.4 \times 10^{-9}$ , and  $4.4 \times 10^{-10}$  mg/kg/day, respectively, as indicated in Figure 3-2.

Several benzidine-congener-derived dyes during dye manufacture and dye use have been tested for possible carcinogenicity. The need for conducting such bioassays was based on the known carcinogenicity of benzidine in humans and the results of cancer epidemiology studies in Japanese kimono painters exposed to benzidine-derived dyes. Also, 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine, benzidine congeners employed in the dye industry, were found to increase tumor incidence in rats (Haley 1975). Five chemicals were evaluated in 2-year carcinogenicity studies: 3,3'-dimethoxybenzidine dihydrochloride (benzidine congener), 3,3'-dimethylbenzidine dihydrochloride (benzidine congener), Direct Blue 15 (a representative 3,3'-dimethoxybenzidine-based dye), Acid Red 114 (a representative 3,3'-dimethylbenzidine-based dye), and Direct Blue 218 (a metallized 3,3'-dihydroxybenzidine-based dye) (NTP 1990, 1991a, 1991b, 1992, 1994). Rats and mice were used in the Direct Blue 218 study, and only rats were used in the other four bioassays. Direct Blue 218 was administered in the feed and the other chemicals in the drinking water. The following brief summary of the results regarding neoplastic lesions has been taken from Morgan et al. (1994). 3,3'-Dimethoxy-

### 3. HEALTH EFFECTS

benzidine, 3,3'-dimethylbenzidine dihydrochloride, Direct Blue 15, and Acid Red 114 all caused a similar spectrum of neoplastic lesions. The primary chemical-related neoplasms were tumors of the skin, Zymbal's gland, oral cavity epithelium, liver, preputial/clitoral glands, and intestines of both males and females. Direct Blue 218 induced an entirely different spectrum of neoplastic lesions. Tumor incidences were not as dramatic, and were seen primarily in the oral cavity epithelium of rats, and in the livers of mice. Morgan et al. (1994) noted that quantitative comparisons of the carcinogenic potencies of the chemicals other than Direct Blue 218 is difficult because of the lack of dose-response relationships, as well as differences in doses and durations of treatments.

#### 3.2.3 Dermal Exposure

##### 3.2.3.1 Death

No studies were located regarding death in humans or animals after dermal exposure to benzidine.

##### 3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, or ocular effects in humans or animals after dermal exposure to benzidine.

**Dermal Effects.** One human case of severe, recurrent, allergic eczematous dermatitis from benzidine use has been reported (Baer 1945), but data on exposure levels were not available. Of 4,600 patients tested over a 5 year period, 231 (5%) showed dermal sensitization to benzidine (Grimalt and Romaguera 1981). Occupational allergic contact dermatitis was clinically diagnosed in 88.5% (208 cases) of such dermally sensitized patients.

No studies were located regarding dermal effects in animals after dermal exposure to benzidine.

##### 3.2.3.3 Immunological and Lymphoreticular Effects

As noted above, allergic contact dermatitis has been observed in workers occupationally exposed to benzidine (Baer 1945; Grimalt and Romaguera 1981). No other studies were located regarding immunological effects in humans or animals after dermal exposure to benzidine.

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No studies were located regarding the following health effects in humans or animals after dermal exposure to benzidine:

#### **3.2.3.4 Neurological Effects**

#### **3.2.3.5 Reproductive Effects**

#### **3.2.3.6 Developmental Effects**

#### **3.2.3.7 Cancer**

No studies were located regarding the carcinogenic effects in humans or animals after dermal exposure to benzidine. Although dermal exposure was identified in many of the occupational studies, the relative contribution to the carcinogenic effect of benzidine could not be quantified.

### **3.3 GENOTOXICITY**

The cytogenetic effects of occupational exposure to benzidine and benzidine-based dyes (Direct Black 38 and Direct Blue 6) were studied in workers at a manufacturing plant in Bulgaria having a recognized high risk of occupational cancer (Mirkova and Lalchev 1990). Twenty-three workers exposed for a mean of 15 years were compared with 30 controls presumed to have had no exposure. A statistically significant (10-fold) increase in the number of circulating peripheral lymphocytes displaying chromosomal aberrations was observed in exposed workers when compared with controls. The highest frequencies of aberrant lymphocytes were associated with the highest airborne dust concentrations of benzidine (0.42–0.86 mg/m<sup>3</sup>) or benzidine-based dyes (7.8–32.3 mg/m<sup>3</sup>), and with the highest mean levels of benzidine found in the urine (1.8–2.3 µg/L). The frequency of polyploid lymphocytes was also elevated in workers when compared with controls. No significant association with smoking was observed. A major strength of this study is the monitoring and biomonitoring of benzidine. These data provide clear evidence of benzidine's genotoxicity in humans under occupational exposure conditions, and are in agreement with oral genotoxicity results from animals and *in vitro* test systems (see below).

Several studies have addressed the *in vivo* genotoxicity of benzidine in animals following oral or parenteral exposure (Table 3-3). Although early studies were conflicting or equivocal, benzidine was clearly demonstrated to induce bone marrow micronuclei in two strains of male mice (C57BL6 and CBA) 24 and 48 hours after a single administration of 300 mg/kg benzidine by oral gavage (Mirkova and Ashby 1988). The number of micronucleated cells per 1,000 normal cells for test groups (5.75–8.75) was

**Table 3-3. Genotoxicity of Benzidine *In Vivo***

Species (test system)	End point	Results	Reference
Nonmammalian cells:			
<i>Drosophila melanogaster</i>	Gene mutation	+	Fahmy and Fahmy 1977
<i>D. melanogaster</i>	Gene mutation	+	Graf et al. 1990
Mammalian cells:			
Rat	Micronucleus test	+	Urwin et al. 1976
Rat	Micronucleus test	–	Trzos et al. 1978
Rat	Micronucleus test	+	Cihak 1979
Mouse	Micronucleus test	+	Tice et al. 1990
Mouse	Micronucleus test	+	Mirkova and Ashby 1988
Mouse	Micronucleus test	+	Mirkova 1990
Mouse	Micronucleus test	–	Harper et al. 1989
Mouse	Micronucleus test	+	Sanderson and Clark 1994
Mouse	Chromosomal aberrations <sup>a</sup>	+	Talaska et al. 1987
Mouse	Chromosomal aberrations	+	Sinsheimer et al. 1992
Mouse	Chromosomal aberrations	+	Das et al. 1994
Mouse	DNA adduct formation	+	Phillips et al. 1990
Rat	DNA damage (alkaline elution)	+	Petzold and Swenberg 1978
Rat	DNA damage (alkaline elution)	+	Parodi et al. 1981
Rat	Unscheduled DNA synthesis	+	Ashby and Mohammed 1988
Rat	Unscheduled DNA synthesis	+	Ashby et al. 1990

<sup>a</sup>Liver cells evaluated in partially hepatectomized mice.

– = negative results; + = positive results; DNA = deoxyribonucleic acid

## 3. HEALTH EFFECTS

3 times that observed in control groups (2.0–2.9). These findings were extended in a subsequent study in which male C57BL6 mice, treated by oral gavage with either a single dose (900 mg/kg) or with three consecutive daily doses (150 or 300 mg/kg), evidenced a positive dose response for bone marrow micronuclei induction (Mirkova 1990). Negative results, however, were reported for a different strain of mice (ICR) treated with single oral gavage doses of 100 or 200 mg benzidine/kg. No significant increases in micronucleated cells were observed in the bone marrow of treated male, female, or pregnant female mice (gestation days 16–17), nor in the livers from the fetuses of treated pregnant female mice (Harper et al. 1989). Noting that adult liver is more sensitive than bone marrow to the chromosome damaging effects of benzidine, and that fetal liver can be more sensitive than adult liver for certain chemicals, the authors had speculated that micronuclei might be detectable in fetal liver. Lower doses, nonoptimum sampling time, and/or strain differences may have contributed to the negative findings in this study. When administered by single oral gavage to male rats, 200 mg benzidine/kg induced unscheduled DNA synthesis in liver cells, which is a repair response to DNA damage (Ashby and Mohammed 1988; Ashby et al. 1990). In a study in mice, intraperitoneal administration of benzidine to pregnant dams increased the frequency of micronucleated polychromatic erythrocytes in the liver of fetuses, which suggested that benzidine (or metabolites) can cross the placenta (Sanderson and Clark 1993).

When tested in many *in vitro* assays (Table 3-4), benzidine has generally tested positive for reverse mutation in *Salmonella typhimurium* in the presence of exogenous metabolic activation (e.g., liver S-9) (Ames et al. 1973; Chung et al. 2000; Dorado and Pueyo 1988; Duverger-van Bogaert et al. 1995; Gregory et al. 1981; Zeiger et al. 1992); negative for SOS DNA repair in *Escherichia coli* (Von der Hude et al. 1988); positive for mutation in yeast (Buchholz et al. 1992; Mitchell and Gilbert 1991); positive (Oberly et al. 1990) or negative (Phillips et al. 1990) for gene mutation in Chinese hamster ovary cells; positive (Fassina et al. 1990; Suter et al. 1992) or negative (O'Donovan 1990; Oglesby et al. 1983) for gene mutation in Chinese hamster V79 cells; and positive (TK locus) or negative (HGPRT locus) for gene mutation in mouse lymphoma cells (Henderson et al. 1990; Myhr and Caspary 1988). Benzidine has also tested positive for chromosome breaks (Svenberg et al. 1976) and sister chromatid exchange (Grady et al. 1986; Lindahl-Kiessling et al. 1989) in cultured human and animal cells; generally positive in cultured hepatocytes for unscheduled DNA synthesis (Kornburst and Barfknecht 1984a, 1984b; Steinmetz et al. 1988; Williams 1978); positive for animal cell transformation (Ashby et al. 1978; Pienta 1980); and negative in cultured mammalian cells (but positive with calf thymus DNA) in the absence of exogenous activation for DNA adduct formation (Phillips et al. 1990).



Table 3-4. Genotoxicity of Benzidine *In Vitro*

Species (test system)	End point	Activation system	Results		Reference
			With activation	Without activation	
Prokaryotic organisms:					
<i>Salmonella typhimurium</i>	Reverse mutation	Human S-9	–	No data	Phillipson and Ioannides 1983
<i>S. typhimurium</i>	Reverse mutation	Human Hep G2	+	No data	Duverger-van Bogaert et al. 1995
<i>S. typhimurium</i>	Reverse mutation	Human S-9	+	–	EPA 1978b
<i>S. typhimurium</i>	Reverse mutation	Human S-9	+	–	Haworth and Lawlor 1978
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	–	Chung et al. 2000
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	–	Ames et al. 1973
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	–	McCann et al. 1975
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	No data	Duverger-van Bogaert et al. 1995
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	–	Garner et al. 1975
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	–	Ferretti et al. 1977
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	–	Anderson and Styles 1978
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	–	Simmon and Shephard 1981
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	–	Messerly et al. 1987
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	No data	Dorado and Pueyo 1988
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	No data	Bos et al. 1983
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	–	No data	Phillipson and Ioannides 1983
<i>S. typhimurium</i>	Reverse mutation	Rat S-9	+	–	Zeiger et al. 1992
<i>S. typhimurium</i>	Reverse mutation	Hamster S-9	+	–	Zeiger et al. 1992
<i>S. typhimurium</i>	Reverse mutation	Modified rat S-9	(+)	No data	Prival and Mitchell 1982

Table 3-4. Genotoxicity of Benzidine *In Vitro* (continued)

Species (test system)	End point	Activation system	Results		Reference
			With activation	Without activation	
<i>S. typhimurium</i>	Reverse mutation	Modified hamster S-9	+	No data	Prival and Mitchell 1982
<i>S. typhimurium</i>	Reverse mutation	Modified hamster ± FMN	+	No data	Prival et al. 1984
<i>S. typhimurium</i>	Reverse mutation	Mouse S-9	+	No data	Phillipson and Ioannides 1983
<i>S. typhimurium</i>	Reverse mutation	Hamster S-9	+	No data	Phillipson and Ioannides 1983
<i>S. typhimurium</i>	Reverse mutation	Pig S-9	–	No data	Phillipson and Ioannides 1983
<i>S. typhimurium</i>	Reverse mutation	Rat hepatocytes	+	No data	Bos et al. 1983
<i>S. typhimurium</i>	Reverse mutation	Bovine bladder urothelial cells	+	No data	Oglesby et al. 1983
<i>S. typhimurium</i>	Reverse mutation	RSV+NAT(B)	+	–	Petry et al. 1988
<i>S. typhimurium</i>	Reverse mutation	RSV–NAT(B)	–	–	Petry et al. 1988
<i>S. typhimurium</i>	Reverse mutation	Rat S-9+NAT(H)	+	–	Grant et al. 1992
<i>S. typhimurium</i>	Reverse mutation	Rat S-9–NAT(H)	–	–	Grant et al. 1992
<i>Escherichia coli</i>	SOS DNA repair	Rat S-9	–	–	Von der Hude et al. 1988
<i>E. coli</i> pol A	DNA repair	Rat S-9	–	–	Fluck et al. 1976
<i>E. coli</i>	SOS DNA repair	Hamster S-9	–	–	Von der Hude et al. 1988
<i>E. coli</i>	Prophase induction	Rat S-9	–	–	Speck et al. 1978
<i>E. coli</i> PQ37	SOS DNA repair	Rat S-9	–	No data	Quillardet et al. 1985
<i>Bacillus subtilis</i>	Rec assay	Rat S-9	(+)	–	Kada 1981
Eukaryotic organisms:					
<i>Streptomyces griseus</i>	Reverse mutation	No data	No data	+	Bucholz et al. 1992
<i>Saccharomyces cerevisiae</i>	Forward mutation	Rat S-9	+	No data	Mitchell and Gilbert 1991

Table 3-4. Genotoxicity of Benzidine *In Vitro* (continued)

Species (test system)	End point	Activation system	Results		Reference
			With activation	Without activation	
Mammalian cells:					
Chinese hamster ovary HGPRT <sup>+</sup> cells	Forward mutation	S-9	–	–	Phillips et al. 1990
Chinese hamster ovary HGPRT <sup>+</sup> cells	Forward mutation	S-9	+	–	Oberly et al. 1990
Chinese hamster V79 HGPRT <sup>+</sup> cells	Forward mutation	Rat S-9	–	–	Fassina et al. 1990
Chinese hamster V79 HGPRT <sup>+</sup> cells	Forward mutation	Mouse S-9	+	–	
Chinese hamster V79 HGPRT <sup>+</sup> cells	Forward mutation	Rat S-9	–	–	O'Donovan 1990
Chinese hamster V79 HGPRT <sup>+</sup> cells	Forward mutation	Rat hepatocytes	–	–	Suter et al. 1992
Chinese hamster V79 PTA <sup>+</sup> cells	Forward mutation	Rat hepatocytes	+	–	Suter et al. 1992
Chinese hamster V79 cells	Forward mutation	Bovine bladder urothelial cells	–	–	Oglesby et al. 1983
L5178YTK <sup>+/-</sup> Mouse lymphoma cells	Forward mutation	Rat S-9	+	No data	Oberly et al. 1984
L5178YTK <sup>+/-</sup> Mouse lymphoma cells	Forward mutation	S-9	+	+	Clay and Cross 1990
L5178YTK <sup>+/-</sup> Mouse lymphoma cells	Forward mutation	Rat S-9	+	+	Riach et al. 1990
L5178YTK <sup>+/-</sup> Mouse lymphoma cells	Forward mutation	Ram PHS	+	(+)	Sarkar et al. 1990
L5178YTK <sup>+/-</sup> Mouse lymphoma cells	Forward mutation	Rat S-9	+	+	Myhr and Caspary 1988
L5178YTK <sup>+/-</sup> Mouse lymphoma cells	Forward mutation	Rat S-9	+	+	Mitchell et al. 1988

Table 3-4. Genotoxicity of Benzidine *In Vitro* (continued)

Species (test system)	End point	Activation system	Results		Reference
			With activation	Without activation	
L5178YTK <sup>+/-</sup> Mouse lymphoma cells	Forward mutation	Rat S-9	+	+	Henderson et al. 1990
L5178Y HGPRT <sup>+</sup> Mouse lymphoma cells	Forward mutation	Rat S-9	-	-	Kennelly et al. 1990
L5178Y HGPRT <sup>+</sup> Mouse lymphoma cells	Forward mutation	Rat S-9	-	-	Henderson et al. 1990
TK6 human lymphoblastoid TK <sup>+/-</sup> cells	Forward mutation	Rat S-9	(+)	(+)	O'Brien et al. 1990
TK6 human lymphoblastoid HGPRT <sup>+</sup> cells	Forward mutation	Rat S-9	-	-	O'Brien et al. 1990
Chinese hamster V79	Chromosome breaks	Rat S-9	+	-	Swenberg et al. 1976
Mouse lymphocytes	Chromosomal aberrations	Mouse S-9	+	No data	Das et al. 1994
H4IIE	Sister chromatid exchange		No data	+	Grady et al. 1986
HEP G2	Sister chromatid exchange		No data	+	Grady et al. 1986
Chinese hamster V79	Sister chromatid exchange		No data	-	Grady et al. 1986
IMR-90	Sister chromatid exchange		No data	-	Grady et al. 1986
Human lymphocytes	Sister chromatid exchange	Rat liver cells	(+)	+	Lindahl-Kiessling et al. 1989
HeLa Cells	Unscheduled DNA synthesis	Rat S-9	+	-	Martin et al. 1978
Rat hepatocytes	Unscheduled DNA synthesis		No data	+	Brouns et al. 1979; Kornbrust and Barfknecht 1984a, 1984b; Williams 1978
Hamster hepatocytes	Unscheduled DNA synthesis		No data	+	Kornbrust and Barfknecht 1984a, 1984b
Rat hepatocytes	Unscheduled DNA synthesis	No data	No data	(+)	Harbach et al. 1991

**Table 3-4. Genotoxicity of Benzidine *In Vitro* (continued)**

Species (test system)	End point	Activation system	Results		Reference
			With activation	Without activation	
Rat hepatocytes	Unscheduled DNA synthesis	No data	No data	+	Steinmetz et al. 1988
Mouse hepatocytes	Unscheduled DNA synthesis	No data	No data	–	Steinmetz et al. 1988
Hamster hepatocytes	Unscheduled DNA synthesis	No data	No data	+	Steinmetz et al. 1988
Monkey hepatocytes	Unscheduled DNA synthesis	No data	No data	–	Steinmetz et al. 1988
Human hepatocytes	Unscheduled DNA synthesis	No data	No data	+	Steinmetz et al. 1988
Human diploid fibroblasts	Unscheduled DNA synthesis	No data	No data	–	Snyder and Matheson 1985
Rat hepatocytes	Unscheduled DNA synthesis	No data	No data	+	Barfknecht et al. 1988
BHK21 C1-13	Cell transformation	Rat S-9	+	–	Ashby et al. 1978
Syrian hamster embryo cells	Cell transformation	No data	No data	+	Pienta 1980
Chinese hamster ovary cells	DNA adduct formation	No data	No data	–	Phillips et al. 1990
Human cervical carcinoma cells	DNA adduct formation	No data	No data	–	Phillips et al. 1990
L5178Y TK <sup>+/–</sup> mouse lymphoma cells	DNA adduct formation	No data	No data	–	Phillips et al. 1990
L5178Y TK <sup>+/–</sup> mouse lymphoma cells	DNA adduct formation	No data	No data	–	Phillips et al. 1990
Mouse lymphoma cells	DNA adduct formation	No data	No data	–	Phillips et al. 1990
Human lymphoblastoid TK6 cells	DNA adduct formation	No data	No data	–	Phillips et al. 1990
Chinese hamster V79 cells	DNA adduct formation	No data	No data	+	Phillips et al. 1990

**Table 3-4. Genotoxicity of Benzidine *In Vitro* (continued)**

Species (test system)	End point	Activation system	Results		Reference
			With activation	Without activation	
Calf thymus DNA	DNA adduct formation	No data	No data	+	Phillips et al. 1990
Calf thymus DNA	DNA adduct formation	RSV	+	-	Petry et al. 1988

- = negative result; + = positive result; (+) = weakly positive result

DNA = deoxyribonucleic acid; FMN = flavin mononucleotide; HGPRT = hypoxanthine guanine phosphoribosyl transferase; NAT(B) = bacterial N-acetyltransferases; NAT(H) = human N-acetyltransferases; PHS = prostaglandin H synthase; PTA = *Pseudomonas* toxin A; RSV = rat seminal vesical microsomes; S-9 = liver homogenate fraction; SOS = inducible bacterial error-prone DNA repair pathway; TK = thymidine kinase

## 3. HEALTH EFFECTS

**3.4 TOXICOKINETICS**

Many studies have been devoted to clarifying the mechanism and etiology of bladder and other cancers caused by benzidine in animals. Both benzidine toxicity and the elimination of benzidine from the body are substantially mediated by metabolic transformation. While some metabolites are detoxication products, others are precursors of proximate and ultimate carcinogens. The latter form adducts with nucleic acids and is presumed to initiate carcinogenesis. The differences in target organs among rodents, dogs, and humans are related to species differences in their metabolic pathways and enzyme activities.

No studies have been reported that indicate that benzidine is absorbed by any process other than passive diffusion. Absorption follows inhalation, oral, and dermal routes of exposure, with the inhalation and dermal routes probably being the most likely for humans. In general, there appears to be a rapid plasma clearance of absorbed benzidine, followed by a more gradual metabolism and clearance of benzidine metabolites (Lakshmi et al. 1990a; Shah and Guthrie 1983). Enterohepatic circulation may contribute to the persistence and toxicity of metabolites of benzidine in the bile (Chipman and Mohn 1989; Percy et al. 1989). Benzidine metabolism involves multiple and complex enzymatic pathways, the relative importance of which may vary according to both species and tissue (Babu et al. 1992, 1993a, 1994a, 1994b; Josephy et al. 1989; Kadlubar et al. 1982; Lakshmi et al. 1990a, 1990b, 1995a, 1995b; Naidu et al. 1992; Wang et al. 1988; Zenser et al. 1996). In the liver, benzidine is thought to be N-acetylated and then N-hydroxylated by the cytochrome P-450 and/or flavin monooxygenase systems, whereas in extrahepatic tissues, peroxidation by prostaglandin H synthase or oxidation by lipoxygenases may play a significant role in benzidine metabolism. Excretion of benzidine, its metabolites, and their conjugates appears approximately equally divided between the urine and the bile/feces (Lakshmi et al. 1990a; Shah and Guthrie 1983).

Some studies also indicate that benzidine-based dyes can be metabolized to benzidine, and that human exposure to the dyes is associated with bladder cancer (Genin 1977; Yoshida and Miyakawa 1973). After oral administration, benzidine dyes can be metabolized to benzidine by microflora of the gastrointestinal tract (Bos et al. 1986). These investigators detected mutagenicity in the urine of ordinary (intestinal microflora-containing), but not germ-free rats after oral administration of two benzidine-based dyes, whereas benzidine produced mutagenic urine in both germ-free and ordinary rats. It is generally agreed that because of the size of the molecule, benzidine-based dyes are unlikely to be absorbed intact; therefore, azo reduction occurs most likely in the intestine and less likely in the liver.

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**3.4.1 Absorption****3.4.1.1 Inhalation Exposure**

No quantitative studies were located regarding absorption in humans or animals after inhalation exposure to benzidine. However, mean urinary levels of benzidine in workers exposed to 0.005–17.6 mg/m<sup>3</sup> benzidine in air increased 4-fold (from 0.01 to 0.04 mg/L) during the course of a workshift, suggesting significant and relatively rapid absorption and excretion. The available data are highly variable, and it is not clear whether high air concentrations correlated with high urinary levels. The relative contribution of dermal or oral absorption was also not rigorously examined in this study (Zavon et al. 1973). Similarly, urine from workers exposed to the dust of Direct Black 38 was found to contain benzidine (2.4–362.5 µg/L), monoacetylbenzidine (6–1,172 µg/L), and diacetylbenzidine (4.2–160 µg/L), suggesting that inhalation absorption had occurred (Dewan et al. 1988; NIOSH 1980c). In addition, it is possible that significant oral exposure to the dyes occurred since inhaled particles are frequently coughed up or brought up by ciliary action and then swallowed. However, the relative contributions of pulmonary and dermal absorption were not discussed in these publications. More recent studies have also provided evidence of inhalation absorption in workers exposed to benzidine (DeMarini et al. 1997; Rothman et al. 1996a).

**3.4.1.2 Oral Exposure**

No quantitative studies were located regarding absorption in humans or animals after oral exposure to benzidine.

Qualitative evidence of absorption after oral administration of <sup>3</sup>H-benzidine to mice in their drinking water was indicated by covalent binding of a benzidine metabolite to DNA in the liver (Martin et al. 1982). A similar experiment with dogs that were fed <sup>3</sup>H-benzidine by capsule resulted in covalent binding to DNA in the epithelium of the bladder (Beland et al. 1983). Another study demonstrated transport of benzidine (but not benzidine-based dyes) across the mucosa of an isolated segment of rat intestine in a perfusion chamber, suggesting that benzidine, but not benzidine-based dyes as such, can be absorbed in the intestines (Bos et al. 1986).



## 3. HEALTH EFFECTS

**3.4.1.3 Dermal Exposure**

It is generally agreed that benzidine bases and salts can be readily absorbed through the intact human skin (Ferber et al. 1976; Meigs et al. 1951, 1954; Zavon et al. 1973). The salts are produced by strong acids acting on a weak base, and consequently, readily hydrolyze in aqueous media to yield some free base at equilibrium. The log<sub>10</sub> octanol-water partition coefficient of the base is reported to be 1.36 (Lu et al. 1977). This is considered sufficiently high to allow significant dermal absorption, particularly during long term exposures or when exposed populations are subject to warm, humid weather and/or strenuous working conditions that result in excessive perspiration.

Levels of benzidine and its metabolites were measured in the urine of industrially exposed workers in March and in August of 1958 (Sciarini and Meigs 1961). During the cooler weather of March, a mean of 5 mg/L of benzidine and its metabolites was reported, compared to a mean of 21.8 mg/L during the warm weather of August. The highest excretion was 31 mg/L in August. Given the conditions observed at this plant, these urinary levels are probably largely due to dermal contact with dust containing benzidine. This conclusion is supported by earlier data showing that daily showers and fresh work clothes reduced the quantity of benzidine and its metabolites excreted in the urine of exposed workers (Meigs et al. 1951). In animals, radioactivity was observed in tissues, urine, and feces following application of 1 mg/kg of radioactive benzidine for 1, 8, and 24 hours to the shaved skin of male F344 rats in a well-controlled study in which the animals were prevented from grooming themselves and licking at the site of benzidine application (Shah and Guthrie 1983). At 24 hours, 49% of the radioactivity was recovered from the skin site, indicating that approximately half of the applied benzidine had penetrated the skin. Since almost 50% of the radioactivity was recovered in the excreta, the results suggested that 50% of the applied dose was absorbed. A preliminary report submitted to EPA under Section 8(e) of the Toxic Substances Control Act (TSCA) stated that nearly all of the benzidine-based dye, Direct Black 38, is absorbed directly through the intact skin of rabbits (International Business Machines 1979). However, this was later attributed to probable ingestion (licking of the skin) (Aldrich 1993). In contrast, Aldrich et al. (1986) applied radiolabeled Direct Black 38 to the shaved dorsal skin of the male F344 rats and New Zealand rabbits that were prevented from licking the site of application, and then measured radioactivity in urine and feces 24–144 hours following dye application. Approximately 3% of the administered radioactivity was detected in the urine and 5% in the feces of rabbits at 144 hours. Excretion of radioactivity was negligible in rats (0.05% in urine and 0.16% in feces). Because skin penetration by the whole dye was considered very unlikely, the absorbed and excreted radioactivity in the rabbits was presumed to represent benzidine liberated by azo-reduction of the dye.

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**3.4.1.4 Other Routes of Exposure**

In an *in vitro* study designed to assess the percutaneous absorption and penetration of  $^{14}\text{C}$ -benzidine in combination with various other chemical mixtures which might be found in occupational settings, isolated perfused porcine skin flaps were topically treated with benzidine+solvent (acetone or DMSO), benzidine+surfactant (sodium lauryl sulfate), benzidine+vasodilator (methyl nicotinate), benzidine+reducing agent ( $\text{SnCl}_2$ ), or various combinations of these mixtures (Baynes et al. 1996). Flow-through diffusion cell systems were also utilized. Absorption was defined as the total amount of radioactivity detected in the perfusate for the entire 8-hour perfusion period. Skin penetration was defined as the sum of total absorption and total radioactivity in the skin. Maximum absorption (and maximum penetration over an 8-hour exposure period) were approximately 3 and 22%, respectively, for a mixture of benzidine+DMSO+surfactant+vasodilator. It was noted that DMSO enhanced absorption more than acetone, and that the reducing agent inhibited benzidine absorption. It was suggested that chemical-biological interactions between the surfactant and vasodilator might enhance benzidine absorption while chemical-chemical interactions between benzidine and  $\text{SnCl}_2$  might inhibit benzidine absorption. The percentage of the dose absorbed in this study is much less than the approximately 50% dermal absorption reported by Shah and Guthrie (1983) in rats *in vivo*.

**3.4.2 Distribution**

There is no information regarding pharmacokinetics of benzidine in children nor is it known whether benzidine can be stored and excreted in breast milk. Although there have been no direct measurements to determine whether it can cross the placenta, there is some indirect evidence that benzidine and/or its metabolites do. This evidence is based on the results of a study in which intraperitoneal administration of benzidine to pregnant mice resulted in the induction of micronuclei in the liver of fetuses (Sanderson and Clark 1993); however, when pregnant mice were orally exposed to benzidine, there was no increase in micronucleated cells either in their livers or the livers of their fetuses (Harper et al. 1989). There is no information on whether benzidine can be stored in maternal tissues and be mobilized during pregnancy or lactation.

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**3.4.2.1 Inhalation Exposure**

No studies were located regarding distribution in humans or animals after inhalation exposure to benzidine.

**3.4.2.2 Oral Exposure**

No studies were located regarding distribution in humans or animals after oral exposure to benzidine.

**3.4.2.3 Dermal Exposure**

No studies were located regarding distribution in humans after dermal exposure to benzidine.

One study was found that reports data on the distribution of benzidine in rats following dermal exposure (Shah and Guthrie 1983). When radiolabeled benzidine was applied to the skin of rats, radioactivity was distributed approximately as follows (percentage of applied radioactivity 1, 8, and 24 hours after application): blood (0.2, 0.3, and 0.7%), liver (1.5, 1.0, and 0.7%), lung (0.09, 0.2, and 0.2%), intestines (1.0, 14.0, and 1.3%), stomach (0.5, 0.4, and 0.08%), carcass (1.9, 4.1, and 6.9%), urine (0.08, 4.1, and 22.8%), and feces (0.01, 0.7, and 18.7%). Twenty-four hours after administration, approximately half of the applied radioactivity remained at the site of application.

**3.4.2.4 Other Routes of Exposure**

Following intravenous injection of 0.2 mg/kg of radiolabeled benzidine, radioactivity was distributed rapidly throughout the body in rats, dogs, and monkeys (Kellner et al. 1973). In rats, substantial activity was found after 4 hours in the lung, the small and large intestines, the bladder, and the kidney, with smaller amounts in all other tissues and fluids examined. Findings were generally similar in dogs, except that there was a 10- to 15-fold higher level of radioactivity in the bladder tissue (consistent with benzidine bladder carcinogenicity in dogs), and much lower activity (about 10% that of rats) in the lung.

Approximately 90% of the radioactivity was cleared from the blood during the first 24 hours after dosing, with the remainder being cleared more slowly. Half-lives of radioactivity in the blood from day 1 to day 6 or 7 were 65 hours in rats and 88 hours in dogs. After 7 days, activity was much reduced in all organs examined from rats, dogs, and monkeys. Highest residual activity was found in the liver for all three species. Expressed as concentration of benzidine in wet tissue, the mean liver value for rats was

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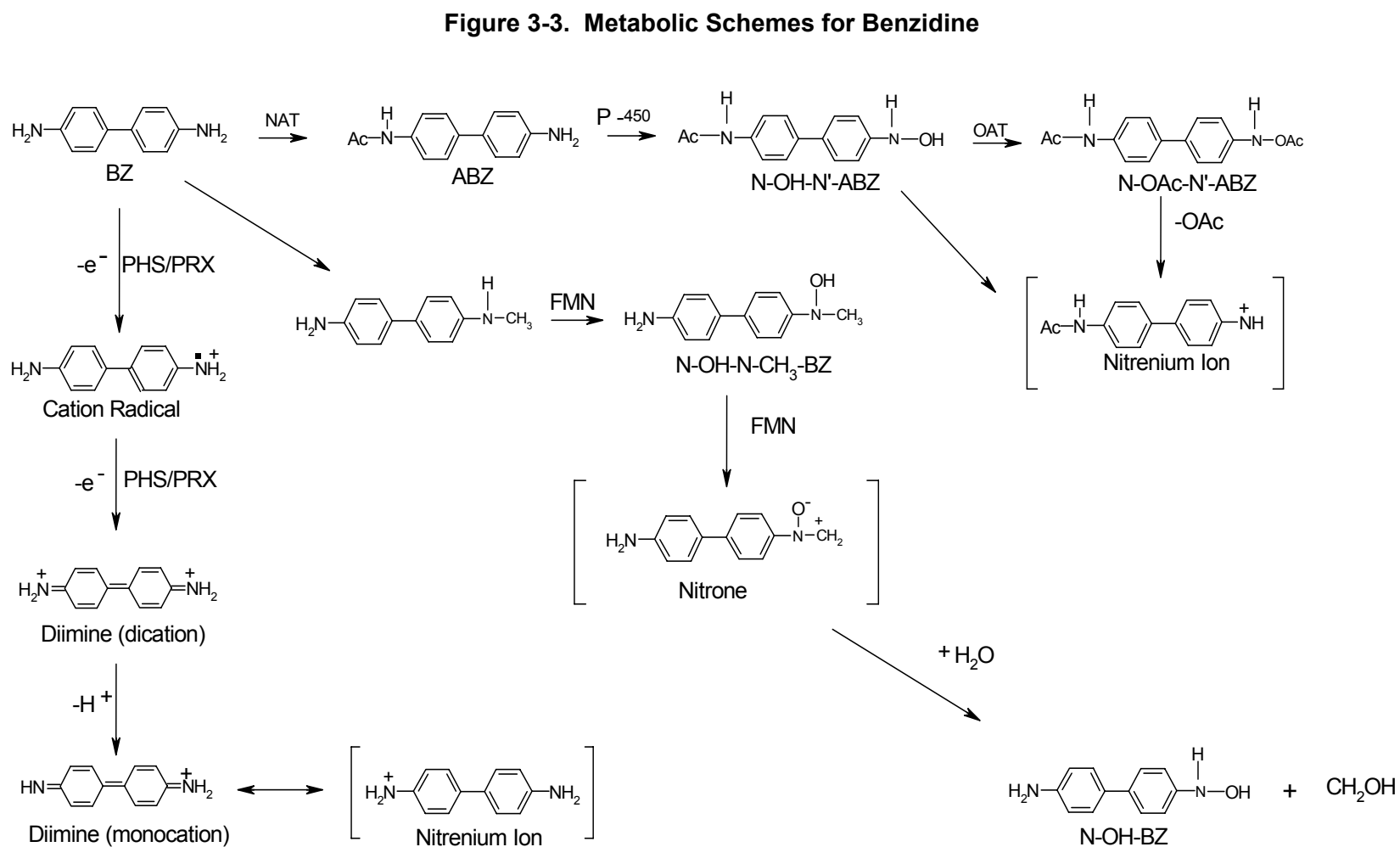
0.042 µg/g, and values were 0.087–0.19 µg/g for three dogs, and 0.01 and 0.027 µg/g for the two monkeys.

Plasma clearance of benzidine in dogs has been found to be fairly rapid (of the amount present 5 minutes after infusion, approximately 10% remained after 5 hours), while metabolism and metabolite clearance occur more gradually. In a study of four dogs over a 5-hour period following intravenous administration of 1 mg/kg radiolabeled benzidine (Lakshmi et al. 1990a), the initial plasma half-life of benzidine was reported to be approximately 30 minutes, while that for total radiolabel (benzidine plus metabolites) was approximately 3 hours. Five hours after infusion, 75% of recovered radioactivity was found collectively in the bile (12–25%), urine (23–52%), and carcass muscle (15–30%). Significant amounts of radioactivity were also detected in fat (3–8%), the liver (4–8%), and plasma (2–7%). Smaller quantities were found in the stomach, intestines, spleen, kidney, heart, and lungs. While the bladder contained the lowest fraction of recovered radioactivity (0.1%), the bladder transitional epithelium exhibited a consistently higher concentration of bound radioactivity than bladder muscle. In liver, kidney, bladder muscle, and bladder epithelium, the majority of radioactivity was bound to protein. Smaller amounts were also bound to DNA. Lynn et al. (1984) performed a similar study in rats after intravenous injection of radiolabeled benzidine. Tissues retaining the most radioactivity after 3 days were muscle and liver. A higher amount was found in the stomach as well. Activity was low in the bladder; this is consistent with the liver, but not the bladder, being a target organ for benzidine-induced carcinogenesis in rats.

The importance of enterohepatic circulation has been demonstrated in both rats and mice, where biliary benzidine and benzidine metabolites can be reabsorbed from the intestines and transported again to the liver (Chipman and Mohn 1989). This enterohepatic recycling contributes to the persistence, the further metabolism, and presumably the hepatotoxicity of benzidine and its metabolites.

### 3.4.3 Metabolism

There is substantial information available from human, animal, and *in vitro* studies regarding various aspects of benzidine metabolism. Numerous *in vivo* metabolites have been reported in a variety of species and a brief discussion of the major ones can be found in earlier EPA documents (EPA 1980a, 1986c) and in more recent publications (Whysner et al. 1996; Zenser et al. 1998). As a general guide, Figure 3-3 presents some of the principal features of several metabolic pathways thought to be involved or potentially involved in the metabolism of benzidine. Although there is no evidence suggesting that the metabolism of benzidine is substantially influenced by route of exposure, there are clear differences in



ABZ = monoacetylbenzidine; BZ = benzidine; FMN = flavin monooxygenase system; N-CH<sub>3</sub>-BZ = N-methylbenzidine; N-OAc-N'-ABZ = N'-acetoxy-N'-acetylbenzidine; N-OH-N'-ABZ = N-hydroxy-N'-acetylbenzidine; N-OH-N-CH<sub>3</sub>-DZ = N-hydroxy-N-methylbenzidine; N-OH-BZ = N-hydroxybenzidine; NAT = N'-acetyltransferase; NMT = N-methyltransferase; Oac = acetoxy; OAT = O-acetyltransferase; P-450 = cytochrome P-450 system; PHS = prostaglandin H synthase; PRX = peroxidase

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metabolism among species or organs. It is not the purpose of this section to present an exhaustive review of all of the studies that investigated the metabolism of benzidine, but to summarize some basic information from the main contributors to this topic and to provide a foundation for the understanding of the mechanism of carcinogenicity of benzidine, which is further discussed in Section 3.5.2.

The urine of humans exposed to benzidine has been reported to contain free benzidine, N-acetylbenzidine, N,N'-diacetylbenzidine, 3-hydroxybenzidine, and 3,3'-dihydroxybenzidine (EPA 1980a, 1986c; Sciarini and Meigs 1961). Following oral or intravenous administration of benzidine to rats, at least 17 metabolites were identified in the urine or bile, principally the mono and di-acetyl derivatives, benzidine-N-glucuronide, N-acetylbenzidine glucuronide, N-hydroxy-N,N'-diacetylbenzidine glucuronide, 3-hydroxy-N,N'-diacetylbenzidine glucuronide, and an N,N'-diacetylbenzidine glutathione conjugate (Lynn et al. 1984). Similar findings were reported using isolated perfused rat liver (Lynn et al. 1983). Results from these and also from more recent studies suggest that three main chemical reactions are involved in the metabolism of benzidine: N-acetylation, N-oxidation, and N-glucuronidation. Because primary amines are easier to oxidize than the acetylated amide products, N-acetylation is considered a detoxification reaction and competes with N-oxidation. Also, because amides do not form glucuronides, N-glucuronidation and N-acetylation are also competing pathways. The prevalence of any one of these reactions is species-specific and will ultimately determine the potential development of organ-specific cancer. These three pathways are discussed below.

N-Acetylation is a reaction that involves the transfer of acetate to the nitrogen or oxygen atom of aromatic amines, hydrazines, and N-hydroxylamines, catalyzed by N-acetyltransferase (NAT), a cytosolic enzyme that exhibits polymorphism. Two NAT isozymes, NAT1 and NAT2, have been reported to differ significantly in their intrinsic stabilities and acceptor substrate selectivity. In several species, including humans, acetylator polymorphism results in rapid, intermediate, and slow acetylator phenotypes. The acetylation of benzidine was studied in liver slices from rats and humans (Lakshmi et al. 1995a) and dogs (Lakshmi et al. 1995b). In rat liver slices incubated for 1 hour with [ $H^3$ ]benzidine, N-acetylbenzidine and N,N'-diacetylbenzidine represented 8.8 and 73% of the total radioactivity recovered, respectively. No unmetabolized benzidine was detected, indicating that rats favor N,N'-diacetylbenzidine formation. In contrast, of the total radioactivity recovered from human liver slices, 19% was benzidine, 34% N'-acetylbenzidine, and only 1.6% N,N'-diacetylbenzidine. When the deacetylase inhibitor paraoxon was included in the experiment with human liver slices, 2% of the radioactivity was recovered as benzidine, 24% as N-acetylbenzidine, and 51% as N,N'-diacetylbenzidine. These findings suggested that

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humans favor N-acetylbenzidine formation, and that a deacetylase influences hepatic metabolism of benzidine more than specific NAT genotype (this issue is further discussed in Sections 3.5.2 and 3.10).

Similar experiments conducted with dog liver slices showed that benzidine was converted to metabolites more polar than benzidine; no acetylated metabolites were detected (Lakshmi et al. 1995b). This suggests that the dog is a nonacetylator with respect to benzidine and, as explained further in Section 3.5.2, this is consistent with the fact that no acetylated DNA adduct was observed in this species. In contrast, N<sup>7</sup>-(deoxyguanosin-8-yl)-N-acetylbenzidine was the major DNA adduct detected following administration of benzidine to rats and mice and of N-acetylbenzidine to hamsters (Kennelly et al. 1984; Martin et al. 1982), in rat liver *in vitro* (Frederick et al. 1985), and in exfoliated urothelial cells of exposed humans (Rothman et al. 1996a). Further studies of the acetylation of benzidine using recombinant NAT1 and NAT2 showed that NAT1 had higher  $K_m$  and  $V_{max}$  values than NAT2 and that N-acetylbenzidine was a preferred substrate for NAT1 (Zenser et al. 1996). Moreover, a higher acetylation ratio was observed in liver slices possessing the NAT1\*10 compared to NAT1\*4 allele, indicating that NAT1 may exhibit a polymorphic expression in human liver.

The NADPH-dependent oxidation of benzidine has been studied by Lakshmi (1996b) who showed that microsomes of  $\beta$ -naphthoflavone-treated rats ( $\beta$ -naphthoflavone is a P-4501A1/1A2 inducer) converted benzidine to 3-hydroxybenzidine. Experiments conducted with inhibitors of cytochrome P-450 (families 1–3) confirmed that benzidine was metabolized by cytochrome P-4501A1/1A2. This metabolite had been found to be the most prominent urinary metabolite in workers exposed to benzidine (Sciarini and Meigs 1961). An earlier study had demonstrated that N-oxidation of N-acetylbenzidine and N,N<sup>7</sup>-diacetylbenzidine occurred in rat and mouse liver subcellular fractions fortified with NADPH and NADH (Frederick et al. 1985). In both species, N-oxidation of N-acetylbenzidine to N<sup>7</sup>-hydroxy-N-acetylbenzidine was faster than the formation of N-hydroxy-N-acetylbenzidine. Further studies by Lakshmi (1996b) showed that both control and  $\beta$ -naphthoflavone-treated rat liver microsomes oxidized N-acetylbenzidine and N,N<sup>7</sup>-diacetylbenzidine and that the relative rate of formation of oxidized compounds was N<sup>7</sup>-hydroxy-N-acetylbenzidine > N-hydroxy-N-acetylbenzidine >> N-hydroxy-N,N<sup>7</sup>-diacetylbenzidine. Lakshmi (1996b) also presented evidence suggesting that P-4501A1 activity was more involved than P-4501A2 and that, under the conditions of the experiment, N-oxidation exceeded ring oxidation.

Glucuronidation is a major metabolic pathway for generating water soluble substances from toxic substances. Glucuronidation of aromatic amines is carried out by UDP-glucuronosyltransferases, which

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are membrane-bound enzymes of the endoplasmic reticulum and that exist in multiple forms. The role of glucuronidation in the metabolism and carcinogenicity of benzidine has been evaluated in numerous studies (Babu et al. 1992, 1993a, 1994a, 1994b, 1995). In the study of Babu et al. (1992), the influence of glucuronidation in the metabolism and carcinogenicity of benzidine was determined using dog liver slices and microsomes. This study demonstrated that the half lives of purified N-benzidine glucuronide in dog urine at 37 EC were 99, 25, and 3 minutes at pH 7.3, 6.3, and 5.3, respectively. It was also shown by Babu et al. (1992) that the N-glucuronide of benzidine was stable in dog plasma at pH 9.3. While the N-glucuronide was found bound to plasma, the binding was weaker than that shown by benzidine. Glucuronide formation of the N-hydroxy metabolites of N-acetylbenzidine (Babu et al. 1995) and N-acetyl-N'-glucuronidation by human, dog, and rat liver (Babu et al. 1993a) was also studied. Human liver slices glucuronidated benzidine and N-acetylbenzidine and the proportion of benzidine and N-acetylbenzidine glucuronidated was affected by the extent of acetylation (Babu et al. 1994a). In contrast to dogs, N-glucuronidation appeared to represent a major pathway for the metabolism of benzidine in humans. Furthermore, results from experiments with inhibitors of glucuronidation in human liver microsomes suggested that more than one UDP-glucuronosyltransferase metabolizes benzidine (Babu et al. 1994a). Recently, the same group of investigators studied the capacity of five different human recombinant UDP-glucuronosyltransferases in COS-1 cells to glucuronide benzidine and its metabolites and found that UGT1A9 exhibited the highest relative rate of metabolism followed by UGT1A4 > UGT1A6 > UGT2B7 > UGT1A1 (Ciotti et al. 2000). UGT1A9 was particularly effective in glucuronidating the two hydroxamic acids, N-hydroxy-N-acetylbenzidine and N-hydroxy-N,N'-diacetylbenzidine, but UGT1A4 showed a 2.3-fold higher rate for N'-hydroxy-N-acetylbenzidine than UGT1A9.

Additional studies by the same group of investigators showed that incubation of N-acetylbenzidine with human liver slices produced a significant amount of N-acetylbenzidine-N'-glucuronide and that rat liver slices needed a much higher concentration of N'-acetylbenzidine to produce the glucuronide (Babu et al. 1993a). Human liver slices incubated with N-acetylbenzidine also formed benzidine (suggesting deacetylation), benzidine glucuronide, and N,N'-diacetylbenzidine (Babu et al. 1994b). Dog liver slices did not produce N'-acetylglucuronide. Liver microsomes from human, rat, and dog produced N-acetylbenzidine-N'-glucuronide and the relative glucuronidation rate of acetylbenzidine was human >> dog > rat. The fact that glucuronidation of N-acetylbenzidine occurred in dog microsomes but not in dog liver slices suggests that dogs rapidly metabolized N-acetylbenzidine, and that deacetylation to benzidine is a major pathway in this species; lack of glucuronidation of N-acetylbenzidine was essentially due in part to lack of substrate.



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Human liver microsomes were also found to glucuronidate N-hydroxymetabolites of N-acetylbenzidine (Babu et al. 1995). The relative rate of glucuronidation was N'-hydroxy-N,N'-diacetylbenzidine >> N'-hydroxy-N-acetylbenzidine = benzidine > N-acetylbenzidine > N-hydroxy-N-acetylbenzidine. At pH 5.5 and 37 °C, the half-lives of the conjugates of N-acetylbenzidine, N'-hydroxy-N-acetylbenzidine, and N-hydroxy-N-acetylbenzidine were 7.5 minutes, 3.5 hours, and 1.8 hours, respectively. Much longer half-lives were obtained for the glucuronides of acetylbenzidine and N'-hydroxy-N-acetylbenzidine when the incubation was carried out at pH 7.4. Compared with N-acetylbenzidine, glucuronides of its N-hydroxymetabolites are more stable at acidic pH. Thus, in acidic urine, it would be more likely that the glucuronide conjugate of N-acetylbenzidine would be hydrolyzed than those of its N-hydroxy-metabolites.

It is not known whether metabolism in adult humans differs qualitatively or quantitatively from metabolism in children or fetuses. However, the expression of two enzyme families (NAT2 and glucuronosyltransferase [UGT]) that contribute to benzidine metabolism is known to vary developmentally (Leeder and Kearns 1997). There is some NAT2 activity present in 16-week-old fetuses. Almost all infants show the slow metabolizer phenotype between birth and the age of two months. NAT2 reaches the adult phenotype distribution in infants at 4–6 months, and by 1–3 years, adult activity is present. For UGT, ontogeny is isozyme-specific, and adult activity is reached at about 6–18 months. The expression of P-450 enzymes varies developmentally as well, but the precise P-450 isozymes involved in metabolism of benzidine are not known.

The relevance of metabolism to the carcinogenic properties of benzidine is summarized in Section 3.5.2.

#### **3.4.4 Elimination and Excretion**

##### **3.4.4.1 Inhalation Exposure**

No quantitative studies were located regarding excretion in humans or animals after inhalation exposure to benzidine. However, excretion of 0–363 µg/L benzidine, 6–1,117 µg/L monoacetylbenzidine, and 4–160 µg/L diacetylbenzidine have been demonstrated in the urine of workers who were potentially exposed to a number of benzidine-based dyes (Dewan et al. 1988; NIOSH 1980c). These dyes can be reduced to free benzidine by azoreductases of the liver and intestinal microflora. Exposure is assumed to have been largely by inhalation, but dermal exposure may also have been significant. Conversely, inhalation exposure was likely a factor in several occupational studies showing urinary excretion where

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dermal exposure to benzidine was reported as the principal route (Meigs et al. 1951, 1954; Sciarini and Meigs 1961).

**3.4.4.2 Oral Exposure**

Information regarding excretion in humans after oral exposure is extremely limited. After a single oral dose of 1.4 mg/kg/day, a human was found to excrete free benzidine, monoacetylbenzidine, and diacetylbenzidine (Engelbertz and Babel 1953), while in another study (Troll et al. 1963), six humans excreted N-hydroxyacetyl amino compounds when dosed with 2.9 mg/kg/day benzidine.

The major route of excretion in rats after a single oral administration of 0.5–50 mg/kg radiolabeled benzidine appeared to be via the feces (Lynn et al. 1984). At the lowest dose studied, 74% of the radiolabel was excreted in the feces during the first 3 days after exposure, as opposed to only 17% in the urine. With increasing dose the percentage of radiolabel excreted in the feces decreased, while that in the urine increased. At the low and mid-level doses (0.5 and 5.0 mg/kg), the major radiolabeled compounds were identified as 3-hydroxy-N,N'-diacetylbenzidine glucuronide (39 and 37%), N,N'-diacetylbenzidine (13 and 17%), N-hydroxy-N,N'-diacetylbenzidine glucuronide (4 and 5%), N-acetylbenzidine (3 and 4%), and free benzidine (2%). As the dose was increased (50 mg/kg), the percentage of N-hydroxy-N,N'-diacetylglucuronide increased substantially (to 24%), largely at the expense of N,N'-diacetylbenzidine (reduced to 4%). No radioactivity was detected in expired air. The analysis of urine and feces after intravenous injection of benzidine confirmed that feces was the main excretory route of radioactivity (Section 3.4.4.4) (Kellner et al. 1973; Lynn et al. 1984).

Oral exposure of monkeys to 10 or 100 mg of benzidine resulted in the urinary excretion of free benzidine and N-acetylbenzidine (Rinde and Troll 1975). The combined 72-hour excretion of these two compounds represented only a small fraction (1.5%) of the administered dose in contrast to the approximately 6% for these two compounds reported by Lynn et al. (1984) in rats.

**3.4.4.3 Dermal Exposure**

Urinary excretion of benzidine and its metabolites was found to occur in humans during workshift exposure, which was determined to be primarily dermal, although inhalation may also have been a contributory route (Meigs et al. 1951, 1954; Sciarini and Meigs 1961). Mean concentration of urinary compounds detected after exposure in the spring were: benzidine (0.28 mg/L), N-acetylbenzidine

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(0.27 mg/L), N,N'-diacetylbenzidine (0.52 mg/L), and conjugated 3-hydroxybenzidine (3.9 mg/L) (Sciarini and Meigs 1961). When determined during hot, humid summer weather, these values increased 1.5- to 5-fold, presumably due to enhanced dermal contact and subsequent absorption. Fecal excretion was not determined.

When radiolabeled benzidine was applied dermally to rats, radiolabel was detected in both the urine and feces as early as 1 hour after treatment (Shah and Guthrie 1983). Excretion was significantly greater (6- to 8-fold) in urine than in feces during the first 8 hours, but by 24 hours, it was virtually the same for both routes (23% in urine, 19% in feces). Identification of the excreted products was not performed.

#### 3.4.4.4 Other Routes of Exposure

The excretion of benzidine after other routes of exposure has been studied in several animal species. Following intravenous exposure of rats to 0.2 or 2.5 mg/kg radiolabeled benzidine, the majority of radiolabel was excreted in the feces during the first 3–7 days (63–80%), as opposed to in the urine (17–29%) (Kellner et al. 1973; Lynn et al. 1984). Experiments with bile duct cannulated rats indicated that virtually all fecal metabolites originate via biliary excretion (Lynn et al. 1984). Urinary metabolites included 3-hydroxy-N,N'-diacetylbenzidine glucuronide (25%), N,N'-diacetylbenzidine (12%), and N-hydroxy-N,N'-diacetylbenzidine glucuronide (4%). Metabolites and levels were similar in bile, except that about half of the 3-hydroxy-N,N'-diacetylbenzidine glucuronide was replaced by the 3-glutathione-N,N'-diacetylbenzidine conjugate.

In contrast to the findings in rat studies, urinary excretion in dogs after intravenous benzidine injection has been reported to range from 1.0 to 2.5 times that found in the bile or feces (Kellner et al. 1973; Lakshmi et al. 1990a) up to about 10 times in an older report (Sciarini and Meigs 1958). About 30% of the radiolabel excreted in the urine or bile was identified as free benzidine. 3-Hydroxybenzidine was a major metabolite (6%) in the bile, but not in the urine, where it apparently appeared as various conjugates. No acetylated metabolites were excreted, in keeping with the lack of N-acetylase activity in dogs. The earlier study reported that urinary concentrations of free benzidine ranged from 2 to 9%, and concentrations of 3-monohydroxybenzidine or its sulfur-conjugate ranged from 25 to 50% (Sciarini and Meigs 1958).

Excretion patterns in three monkeys following intravenous injections of 0.2 mg/kg benzidine varied too substantially to make generalizations (Kellner et al. 1973). Cumulative excretion during the first 7 days

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varied from 30 to 70% in the urine, and from 5 to 36% in the feces. Thin-layer chromatography of urine samples was thought to indicate the presence of N-acetylated benzidine metabolites; however, these were not identified.

Intraperitoneal injection of benzidine in rats led to the biliary excretion of bioactivated metabolites as indicated by the bile's induction of nuclear anomalies in intestinal epithelial cells (Percy et al. 1989), or bacterial mutagenicity in a host-mediated assay (Chipman and Mohn 1989). Following intraperitoneal injection of mice with 100 mg/kg benzidine, benzidine and its metabolites were found in the urine in the following percentages: free benzidine (10%), N-acetylbenzidine (3.4%), N,N'-diacetylbenzidine (2.6%), 3-hydroxy ethereal sulfate (29%), 3-hydroxy-benzidine glucuronide (12%), N-hydrogen sulfate or glucuronide conjugates (18%), and monoacetylated 3-hydroxy ethereal sulfate or glucuronide benzidine conjugates (25%). Fecal excretion was not monitored (Sciarini and Meigs 1958).

#### **3.4.5 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 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.

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 et al. 1987; Andersen and Krishnan 1994). 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.

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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 3-4 shows a conceptualized representation of a PBPK model.

No PBPK modeling studies were located for benzidine.

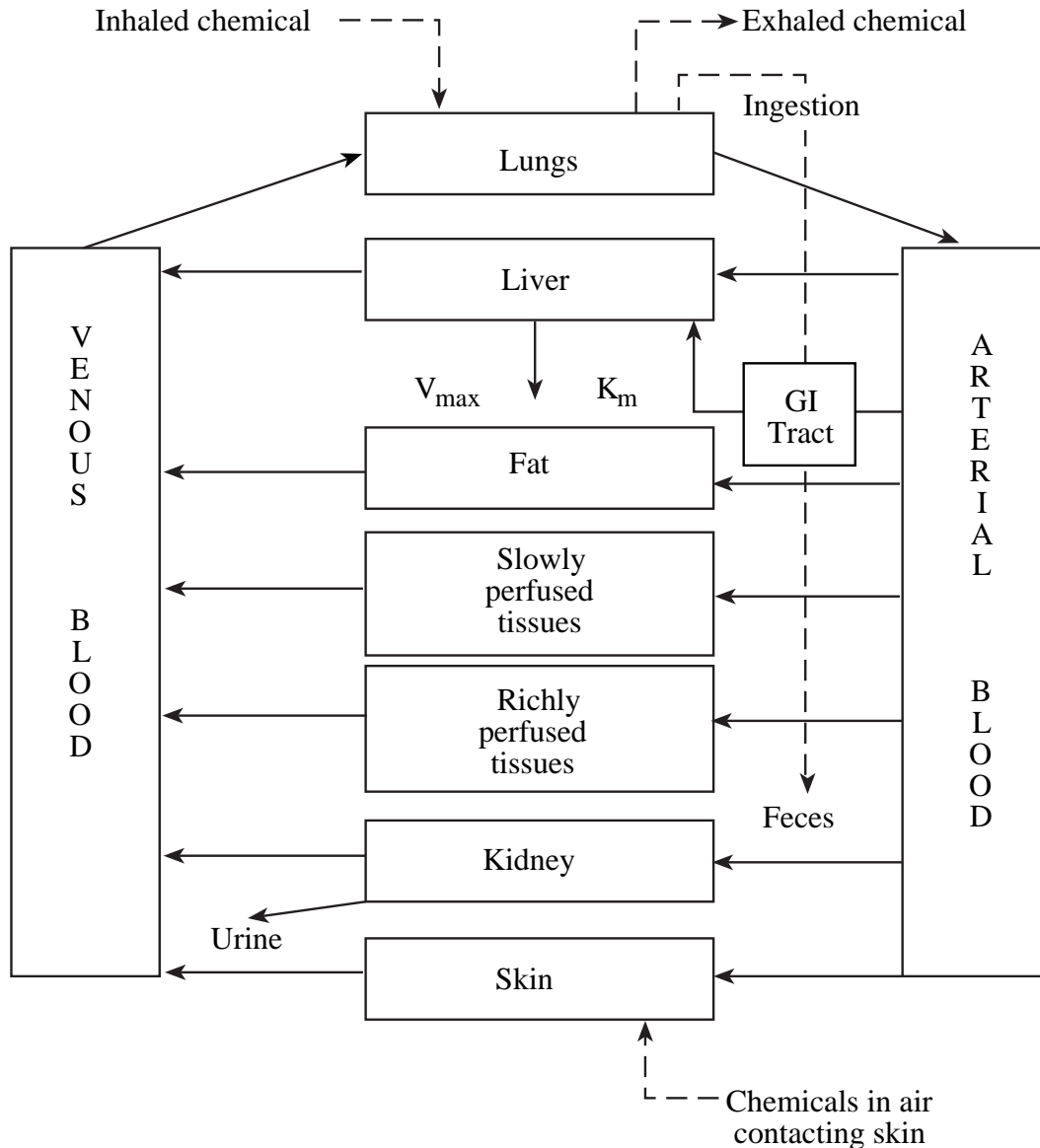
## 3.5 MECHANISMS OF ACTION

### 3.5.1 Pharmacokinetic Mechanisms

There have been no reports that provide any data that benzidine is absorbed by any process other than passive diffusion, regardless of the route of exposure. Benzidine appears readily absorbed across the

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**Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance**



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.

Source: adapted from Krishnan et al. 1994

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intestinal mucosa (Bos et al. 1986), through the skin (Aldrich et al. 1986; Meigs et al. 1951; Shah and Guthrie 1983), and probably by pulmonary tissue as well (Zavon et al. 1973).

Benzidine and/or its metabolites are distributed throughout most of the body after dermal exposure in rats (Shah and Guthrie 1983), and after injection in rats and dogs (Kellner et al. 1973). No evidence has been reported to indicate that distribution is mediated by any carrier or binding protein, although conjugation of partially bioactivated benzidine metabolites with glucuronide may aid in their transport to target sites (e.g., from liver to bladder). In dogs administered benzidine intravenously, benzidine-glucuronide was a major metabolite in plasma (Babu et al. 1992). There is evidence in rats (patterns of genotoxic effects induced in the intestine by bile) to suggest that enterohepatic circulation may be involved in the persistence and/or further bioactivation of benzidine reactive metabolites (Chipman and Mohn 1989; Percy et al. 1989).

#### 3.5.2 Mechanisms of Toxicity

Cancer is the principal and best documented toxic effect of benzidine in both humans (Bi et al. 1992; Bulbulyan et al. 1995; Delzell et al. 1989; Goldwater et al. 1965; Mancusco and El-Attar 1967; Mason et al. 1986; Montanaro et al. 1997; Naito et al. 1995; Piolatto et al. 1991; Shinka et al. 1991; Wu 1988; Xue-Yun et al. 1990; Zavon et al. 1973) and animals (Bonser 1959; Frith et al. 1979, 1980; Griswold et al. 1968; Littlefield et al. 1984; Nelson et al. 1982; Saffiotti et al. 1967; Schieferstein 1982; Spitz et al. 1950; Vesselinovitch et al. 1975; Vorce and Goodman 1989; Zabezhinskii 1970). Like many other arylamines, benzidine is postulated to require metabolic activation to electrophilic derivatives in order to manifest its carcinogenicity and genotoxicity through covalent binding with DNA (Beland et al. 1983; Brouns et al. 1982; Iba 1987; Lang and Iba 1988). One metabolic scheme for bladder cancer, summarized by Wang et al. (1990), involves N-acetylation, N-hydroxylation in the liver, transport of the glucuronide conjugate of the resulting intermediate to the bladder, then hydrolysis of the conjugate followed by absorption and ultimate activation via O-acetylation. This scheme was based on results from experiments in rats with implanted heterotrophic bladders (Wang et al. 1990). In order to circumvent systemic circulation and nonbladder-associated metabolism, benzidine and several metabolites were instilled once a week for 20 weeks directly into these heterotrophic bladders. Heterotrophic bladder cancer was observed in 1 of 39 control animals, 1 of 29 treated with benzidine, 18 of 30 treated with N'-hydroxy-N-acetylbenzidine, and in all 28 treated with N'-hydroxy-N-acetylbenzidine N'-glucuronide. This, however, did not entirely explain the fact that benzidine induces primarily liver cancer in rats, whereas dogs and humans develop bladder cancer as a result of exposure to benzidine. Studies by Zenser and coworkers have provided a

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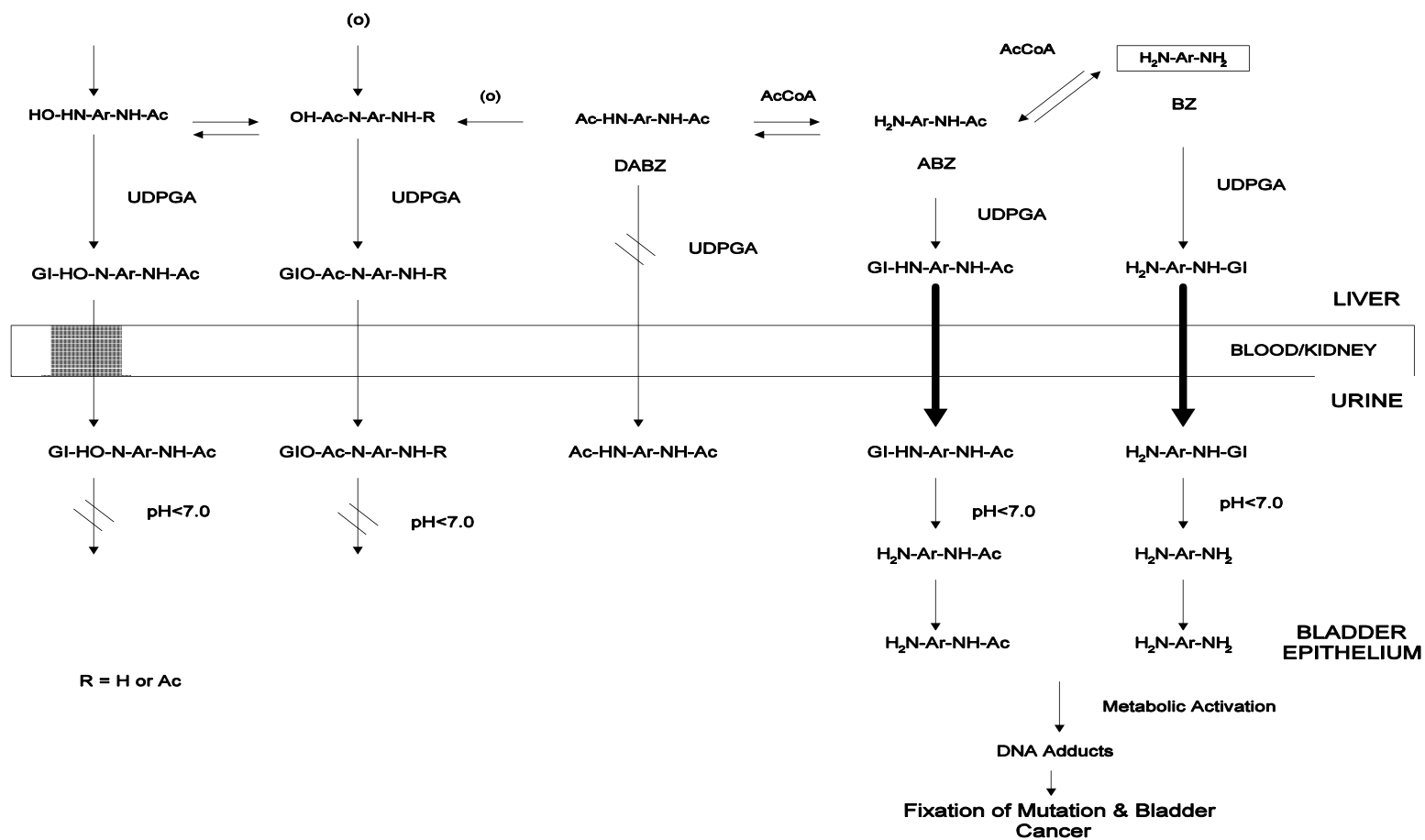
hypothesis that is supported by experimental data. Based on the fact that rats favor acetylation of benzidine and N-acetylbenzidine (Lakshmi et al. 1995a), it was proposed that N-acetylated-N'-glucuronide would not accumulate in rat urine because (1) little N-acetylbenzidine is available and (2) rat hepatic UDP-glucuronosyltransferase activity for N-acetylbenzidine is much lower than in humans. Transient increases in hepatic N-acetylbenzidine that might occur because deacetylation could lead to N-oxidation to hydroxylamine, or N,N'-diacetylbenzidine could undergo N-oxidation and subsequent O-acetylation or N,O-transacetylation to yield mono or diacetylated DNA adducts seen in rat liver. Undetectable or low levels of urinary benzidine or N-acetylbenzidine and/or their glucuronides is consistent with the low incidence of bladder cancer in rats. Results from Morton et al. (1980) demonstrated that in rodents, N-hydroxy-N,N'-diacetylbenzidine can be esterified to an electrophilic reactant by hepatic sulfotransferases and suggested the involvement of this metabolite in the liver carcinogenicity of benzidine.

In humans, Lakshmi et al. (1995a) proposed that benzidine and N-acetylbenzidine are glucuronidated in the liver and transported to the bladder lumen where they are hydrolyzed by acidic urine. Activation in the bladder could include peroxidation by prostaglandin H synthetase (Flammang et al. 1989; Wise et al. 1984c), oxidation by cytochrome P-450 (Butler et al. 1989), and O-esterification by O-acetyltransferase (Frederickson et al. 1992), or N,O-acetyltransferase (Hatcher et al. 1992). N-Acetylbenzidine can be further hydroxylated before glucuronidation. Dogs are nonacetylators, but their ability to form benzidine-N-glucuronide (Babu et al. 1992, 1993a) may contribute to the high incidence of bladder rather than liver cancer. The glucuronides of benzidine and N-acetylbenzidine were found to be much more stable at pH 7.4 than 5.3 (Babu et al. 1992, 1995). The proposed role of metabolism in benzidine-induced cancer in liver and urinary bladder is presented in Figure 3-5; this figure has been adapted from Babu et al. (1995).

Regardless of the pathway involved, DNA adducts with N-acetylated benzidine derivatives have been observed in rodents and humans, principally that of N-(deoxyguanosin-8-yl)-N'-acetylbenzidine (Kennelly et al. 1984; Martin et al. 1982; Rothman et al. 1996a; Yamazoe et al. 1988). This DNA adduct is presumed to be formed by O-acetylation of N'-hydroxy-N-acetylbenzidine and subsequent binding to a DNA base. As previously mentioned, acidic urine is thought to release the amine from the glucuronides; the amine can then be activated, for example, by prostaglandin H synthase to initiate carcinogenesis. The predominant reactive intermediate generated by prostaglandin H synthase and related peroxidases has been identified as benzidinediimine, and it reacts primarily with the C8 position of deoxyguanosine (Yamazoe et al. 1988). Lakshmi et al. (1994) suggested that at acid pH, benzidinediimine is in



Figure 3-5. The Proposed Role of Metabolism in Benzidine-Induced Cancer



ABZ = N'-acetylbenzidine, AcCoA = Acetylcoenzyme A, BZ = Benzidine, DABZ = N,N'-diacetylbenzidine, HO-HN-Ar-NH-Ac = N'-hydroxy-N-acetylbenzidine, OH-Ac-N-Ar-NH-R = N-hydroxy-N-acetylbenzidine, UDPGA = UDP-glucuronic acid

Source: Babu et al. (1995)

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equilibrium with nitrenium ion, the species proposed to bind to bladder DNA. Furthermore, they showed that the *in vitro* formation of benzidinediimine could be prevented by conjugation with glutathione to generate 3-(glutathion-S-yl)-benzidine. The formation of the conjugate was dependent upon diimine and not benzidine, suggesting that conversion of benzidine to diimine occurs before formation of the conjugate.

Susceptibility to bladder cancer has been linked to the slow acetylator type of the polymorphic NAT2 N-acetyltransferase gene (Blum et al. 1991; Ohsako and Deguchi 1990). A study of Chinese workers with high exposure to benzidine demonstrated a 100-fold increased risk for bladder cancer (Bi et al. 1992). However, when members of the benzidine-exposed cohort were screened for their acetylator phenotype and genotype, no positive association was found between N-acetylation phenotype or genotype and bladder cancer risk (Hayes et al. 1993). The authors suggested that the difference between their results and those of the previous investigators may be due to the fact that their subjects were only exposed to benzidine, while those in earlier studies were also exposed to other aromatic amines (monoamines). This was consistent with the fact that N-acetylamines undergo much less oxidation than the monoamine; therefore, for monoamines, acetylation is a detoxification reaction. In contrast, N-acetylbenzidine is relatively reactive and still susceptible to N'-oxidation or N'-glucuronidation; thus, acetylation of benzidine is an activation process potentially leading to bladder cancer. Further research conducted in recent years has shown that slow acetylators are not at increased risk for bladder cancer relative to fast acetylators (Lakshmi et al. 1995a; Rothman et al. 1996a; Zenser et al. 1996). These data are consistent with the observation that benzidine can be acetylated by NAT1 for which genotypic differences in acetylation rate have not been observed. NAT1 in humans may have a dominant role in acetylation of benzidine (Zenser et al. 1996). In addition, the deacylase could have a dominant effect on the profile of benzidine metabolites formed (Lakshmi et al. 1995a). These studies are discussed in more detail in Section 3.10.

The genotoxicity of benzidine is also mediated through bioactivated, electrophilic metabolites. This has been shown in: mutagenicity assays in many prokaryotic and eukaryotic systems (Buchholz et al. 1992; Dorado and Pueyo 1988; Duverger-van Bogaert et al. 1995; Henderson et al. 1990; Myhr and Caspary 1988; Sarkar et al. 1990; Zeiger et al. 1992); *in vivo* and *in vitro* unscheduled DNA synthesis tests (Ashby et al. 1990; Steinmetz et al. 1988); chromosomal aberrations assays (Das et al. 1994; Sinsheimer et al. 1992), assays for micronucleus formation (Harper et al. 1989; Mirkova and Ashby 1988; Sanderson and Clark 1993; Tice et al. 1990); and in tests for sister chromatid exchanges (Lindhahl-Kiessling et al. 1989). Benzidine metabolites also bind to protein and RNA (Kadlubar et al. 1986b; Lakshmi et al. 1990a; Zenser

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et al. 1983), and hemoglobin (Birner et al. 1990; Zwirner-Baier and Neumann 1998), although specific toxicological responses are not yet attributable to these effects.

When male mice were administered 86 ppm benzidine as benzidine dihydrochloride in drinking water for 1 year, 13 of 22 (59%) observed liver tumors were found to contain a mutation in codon 61 of the *H-ras* oncogene (Fox 1990). Although a similar frequency of oncogene mutations were observed in spontaneous liver tumors, much lower frequencies (7–21%) were observed in tumors induced by several nongenotoxic agents. These results are consistent with genotoxicity being at least a component of benzidine's observed carcinogenicity. Epigenetic mechanisms may also be involved, however, as *H-ras* and *K-ras* oncogenes in benzidine-induced liver tumors were found to be hypomethylated in comparison to adjacent nontumor tissue (Vorce and Goodman 1989). Hypomethylation of a gene is thought to enhance its transcription, and thus, benzidine may be capable of facilitating aberrant expression of genes that are involved in carcinogenesis.

#### 3.5.3 Animal-to-Human Extrapolations

Relatively little information is available on noncancer effects of benzidine. Therefore, an attempt to discuss potential interspecies differences or similarities in benzidine noncancer toxicity based on the limited information available is speculative at this time. Benzidine is a bladder carcinogen in humans and dogs, and primarily a liver carcinogen in rodents. The differences in target site are attributed to the species-specific metabolic pathways for activation of benzidine to a reactive intermediate. Extrapolation of carcinogenicity data for benzidine from any one animal species to humans is inappropriate unless there is information suggesting that the particular animal species and humans share common metabolic pathways that produce similar carcinogenic chemical species.

### 3.6 ENDOCRINE DISRUPTION

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones, or otherwise interfere with the normal function of the endocrine system. Chemicals with this type of activity are most commonly referred to as endocrine disruptors. Some scientists believe that chemicals with the ability to disrupt the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. Others believe that endocrine disrupting chemicals do not pose a significant health risk, particularly in light of the fact that hormone mimics exist in the natural environment. Examples of natural hormone

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mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These compounds are derived from plants and are similar in structure and action as endogenous estrogen. While there is some controversy over the public health significance of endocrine disrupting chemicals, it is agreed that the potential exists for these compounds to affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior (EPA 1997). As a result, endocrine disruptors may play a role in the disruption of sexual function, immune suppression, and neurobehavioral function. Endocrine disruption is also thought to be involved in the induction of breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No studies were located regarding endocrine disruption in humans or animals after exposure to benzidine.

#### 3.7 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 resulting from 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 6.6 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 prenatal and postnatal 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

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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. 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 et al. 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 newborns who all have a low glomerular filtration rate and have 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 remaining 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, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

There are no studies that specifically addressed the health effects of exposure to benzidine in children or immature animals; therefore, it is unknown whether children differ from adults in their susceptibility to health effects from benzidine. Data in adults are mostly derived from occupational studies and, despite the inherent limitations of this type of study, there is unequivocal evidence that benzidine is a bladder carcinogen in humans (Bi et al. 1992; Ferber et al. 1976; Goldwater et al. 1965; Mancuso and El-Attar 1967; Mason et al. 1986; Meigs et al. 1986; Shinka et al. 1991; Tsuchiya et al. 1975; Vigliani and Barsotti 1962; Xue-Yun et al. 1990; Zavan et al. 1973). There is also suggestive evidence of carcinogenicity at other tissue sites as well (Delzell et al. 1989; Morinaga et al. 1982; Okubo et al. 1985; Piolatto et al. 1991; Wu 1988; Zavan et al. 1973).

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The only information regarding possible adverse developmental effects in humans is that provided by a study by Wilkins and Sinks (1990) which suggested an association between parental exposure to benzidine and childhood brain tumors. However, results and conclusions of this study are largely speculative due to lack of sufficient validation of the job exposure matrix used to estimate exposure levels. Also, a somewhat limited epidemiological study failed to detect any evidence of elevated rates for birth defects in residents living near a Superfund site contaminated with benzidine (Budnick et al. 1984). However, the extent of actual exposure (if any) to benzidine was not determined. There is considerable evidence that benzidine or its metabolites can be genotoxic, but it is unknown whether parental exposure could result in benzidine metabolites reaching parental germ cells, or whether this might affect childhood development or cancer incidence.

There is no information regarding pharmacokinetics of benzidine in children nor it is known whether benzidine can be stored and excreted in breast milk. Although there have been no direct measurements to determine whether benzidine 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 intraperitoneal administration of benzidine to pregnant mice resulted in the induction of micronuclei in the liver of fetuses (Sanderson and Clark 1993); however, when pregnant mice were orally exposed to benzidine, there was no increase in micronucleated cells either in their livers or the livers of their fetuses (Harper et al. 1989). There is no information on whether benzidine can be stored in maternal tissues and be mobilized during pregnancy or lactation.

There is no information on the metabolism of benzidine in children. Analysis of urine and bile from humans exposed to benzidine revealed the presence of metabolites derived from phase I and phase II enzymatic reactions (EPA 1980a, 1986c; Sciarini and Meigs 1961). Biotransformation of benzidine begins with N-acetylation. 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 Kerns 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 1–3 years of age. Results from earlier studies had suggested that humans possessing a slow acetylator phenotype may be at a higher risk for developing bladder cancer (Hanke and Krajewska 1990). However, more recent data suggest that the acetylation rate may not be an important risk factor (Hayes et al. 1993; Rothman et al. 1996a). It was also shown that in humans, NAT1 rather than NAT2 plays a more important role in the acetylation of both benzidine and N-acetylbenzidine (Zenser et al. 1996). UGT also

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contributes to benzidine metabolism and is known to vary developmentally as well (Leeder and Kearns 1997). Ontogeny is isozyme-specific and adult activity is reached at the age of 6-18 months.

It is generally accepted that benzidine, like many other arylamines, requires metabolic activation by the cytochrome P-450 system in order to manifest genotoxicity and carcinogenicity. The expression of some P-450 enzymes does vary developmentally (Leeder and Kearns 1997). However, for benzidine, the precise cytochrome P-450 isozymes involved in its metabolism by humans are not known.

There are no biomarkers of exposure or effect for benzidine that have been validated in children or in adults exposed as children. No studies were located regarding interactions of benzidine with other chemicals in children or adults.

No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to benzidine, 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 benzidine in adults might be contraindicated in children.

#### **3.8 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 (NAS/NRC 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 (NAS/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

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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 benzidine are discussed in Section 3.8.1.

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 benzidine are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 "Populations That Are Unusually Susceptible".

#### **3.8.1 Biomarkers Used to Identify or Quantify Exposure to Benzidine**

As indicated by a number of studies previously discussed in Section 3.4.3 (Meigs et al. 1951, 1954; Sciarini and Meigs 1961), a variety of benzidine compounds can be detected in the urine of humans exposed to benzidine or benzidine-based dyes. These include benzidine, hydroxybenzidine, N-acetylbenzidine, and N,N'-diacetylbenzidine, and various glucuronic acid conjugates of these compounds. For example, Dewan et al. (1988) used a high performance liquid chromatography instrument equipped with an absorbance detector to monitor benzidine and its mono- and diacetylated derivatives in the urine of workers exposed at a dye-manufacturing facility. Detection limits were 0.25 µg/L for the former two compounds, and 0.5 µg/L for the latter. These biomarkers are specific for benzidine or benzidine-derived compounds, and can be confidently identified with the use of appropriate standards, especially in situations where exposure to such compounds seems reasonable. Although there are no background levels of such biomarker compounds, their urinary levels are likely to fall below detection limits within several days to 2 weeks due to relatively short biological half-lives.



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With respect to biomarkers that can indicate or quantify exposure, covalent binding of benzidine and some of its congeners to hemoglobin has been demonstrated as a promising approach (Birner et al. 1990; Neumann 1988; Zwirner-Baier and Neumann 1998). After oral treatment of rats with 142 mg/kg of benzidine, hemoglobin adducts were hydrolyzed under alkaline conditions, extracted, and then analyzed by high performance liquid chromatography with an electrochemical detector (Birner et al. 1990). The method is based on the cleavage of sulfinic acid amides formed *in vivo* with hemoglobin by the reaction of metabolically generated nitrosoarenes and the SH-group of cysteine. With benzidine, three cleavage products were identified: N-acetylbenzidine (the major component), benzidine itself, and 4-aminobiphenyl (suggesting the occurrence of a previously unknown metabolic pathway for benzidine). The authors proposed using the analysis of hemoglobin adducts in human blood to monitor for exposure to carcinogenic compounds such as benzidine. The same cleavage products (benzidine, N-acetylbenzidine, and 4-aminobiphenyl) were found in a ratio of 1:17:1 in a more recent study by the oral route in rats (Zwirner-Baier and Neumann 1998).

Adducts of benzidine and its metabolites with DNA can also be an extremely sensitive biomarker for detecting and quantifying exposure (Levy and Weber 1988). Hepatic DNA was isolated from mice injected intraperitoneally with 90 mg/kg of benzidine, hydrolyzed, and then solvent-treated to enhance the relative concentration of nucleotide-benzidine adducts. Following  $^{32}\text{P}$  postlabelling, these adducts were resolved by reverse-phase ion-pair high performance liquid chromatography. The major benzidine adduct appeared to be acetylated. This method detected adducts after as little as 3 hours of exposure, and required less than 1  $\mu\text{g}$  of mouse hepatic DNA. A similar approach has detected attomoles ( $10^{-18}$  moles) of benzidine-DNA adduct in human peripheral lymphocytes exposed *in vitro* to benzidine (Gupta et al. 1988). The use of standards and controls are important to this methodology of DNA adduct characterization, which is extremely sensitive and relatively rapid. Benzidine-DNA adducts have also been found *in vivo* in peripheral white blood cells (WBC) and exfoliated urothelial cells of workers occupationally exposed to benzidine (Rothman et al. 1996a; Zhou et al. 1997). The most predominant adduct (N-acetylated) co-chromatographed with a synthetic N-(3'-phosphodeoxyguanosin-8-yl)-N'-acetylbenzidine standard; the median level (range) of this adduct in WBC DNA was 194.4 (3.2–975) RAL (relative adduct labeling)  $\times 10^9$  in exposed workers and 1.4 (0.1–6.4) in the control subjects. There was a striking correlation between levels of this DNA adduct in WBCs and exfoliated urothelial cells; additionally, the combined levels of urinary benzidine, N-acetylbenzidine, and N,N'-diacetylbenzidine correlated with the levels of the major DNA adduct found in both WBCs and exfoliated urothelial cells (Zhou et al. 1997). A recent study reported that the mutagenicity of urine from workers to *Salmonella* strain YG1024 was exposure related (DeMarini et al. 1997). That is, the mean urinary mutagenicity

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(revertants/ $\mu$ mol creatinine) of unexposed controls was 2.8 compared to 8.2 in low-exposure workers and 123 in high-exposure workers. In addition, urinary mutagenicity showed a strong positive correlation with benzidine urinary metabolites and the level of the presumptive urothelial DNA adduct. Adducts of benzidine with either hemoglobin or DNA are specific for exposure to benzidine or to any substance that, as a result of biotransformation in the body, may give origin to benzidine.

The possibility that mutations in specific genes can be used as biomarkers of exposure (and/or effect) to benzidine (and other arylamines) has also been examined. For example, Taylor et al. (1996) compared the frequency and pattern of p53 (a well-known tumor suppressor gene) mutations in 34 bladder tumors from American workers with past high-level occupational exposure to arylamines to those in 30 bladder tumors from people without such exposure. Their analysis revealed no differences for p53 mutations between the two groups suggesting that exposure to arylamines does not leave a mutational "footprint" in the p53 gene. In contrast, Yasunaga et al. (1997) found that bladder lesions from 26 Japanese workers occupationally exposed to aromatic amines exhibited different patterns of p53 mutations from nonoccupational bladder lesions. Cytosine to thymidine transitions were the most common mutations. The reason for this discrepancy is not apparent. The results of Yasunaga et al. (1997) also suggested that the multifocality noted in most occupational bladder cancer arises both from multiple clonal lesions and from the dissemination of a single clone. Sorlie et al. (1998) analyzed the pattern of mutations in four genes (p53, p16<sup>MTS1</sup>, p21<sup>WAF1</sup>, and H-*ras*) in 21 cases of bladder cancer among western European workers exposed to aromatic amines and found that only p53 had a high frequency of mutations. The spectrum of mutations found was highly suggestive of an involvement of exogenous carcinogens and was identical to the spectrum of p53 mutations detected in bladder cancers of the general population. In the exposed workers, p53 mutations were associated with tumor grade and with high occupational and tobacco exposure. The data further suggested that the same carcinogens may be responsible for the development of bladder cancer in workers exposed to aromatic amines and in the general population. An additional study of workers exposed to 2-naphthylamine and benzidine reported over expression of the *ras* oncogene in bladder biopsies relative to unexposed cancer patients, patients with benign conditions of the bladder, and healthy subjects (Novara et al. 1996). c-erbB-2 (an oncogene whose expression is associated with invasiveness and prognosis) was over expressed in both unexposed and exposed bladder cancer cases and, therefore, was not associated with occupational exposure.

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**3.8.2 Biomarkers Used to Characterize Effects Caused by Benzidine**

Early studies (Meigs et al. 1986; Vigliani and Barsotti 1962; Zavon et al. 1973) used bloody urine and cytology as a biomarker for observation of bladder carcinomas. More recent studies have focused on the binding of benzidine's activated metabolites to cellular macromolecules. As discussed in the previous section, hemoglobin and DNA adducts derived from benzidine and its metabolites have been identified and quantified in cells of the blood and liver. Following intravenous administration of radiolabeled benzidine in dogs, significant amounts of DNA binding were observed in the liver, kidney, and bladder (Lakshmi et al. 1990a). The highest concentration of binding was found in the bladder transitional epithelium, the principal target tissue for benzidine's carcinogenic action in this species. DNA adducts were also identified and quantified in bladder epithelial and liver tissues of dogs exposed orally to benzidine and N-acetylbenzidine (Beland et al. 1983). Arylamine substitution at the C8 position of deoxyguanosine was the dominant adduct, and the level of adduct formation correlated with the bacterial mutagenicity of these compounds.

As evidence of genotoxicity, human exposure to benzidine and benzidine-based dyes has been shown to induce chromosomal aberrations and polyploidy in circulating peripheral lymphocytes (Mirkova and Lalchev 1990). Mutagenicity has also been detected in the urine and bile of rodents treated with benzidine (Bos et al. 1980, 1981; Chipman and Mohn 1989). The formation of nuclear anomalies (micronuclei, pyknotic, and karyorrhectic nuclei) in intestinal epithelial cells of rats treated with benzidine is consistent with its intestinal carcinogenic effect in this species (Percy et al. 1989), as is the induction of DNA repair synthesis in explanted rabbit bladder tissue exposed to benzidine (McQueen et al. 1987). However, these and other manifestations of benzidine's genotoxicity are not specific to benzidine or even arylamine exposure.

The liver cytosol activity of arylsulfotransferase was found to be reduced in rats treated with 120 ppm of benzidine in drinking water (approximately 0.26 mg/kg/day) (Ringer and Norton 1988). In rats, this pattern may be characteristic of, though not unique to, liver carcinogens which can utilize the arylsulfotransferase pathway for bioactivation. Its sensitivity, relative specificity, and relevance as a human biomarker are not currently known. The same uncertainties exist for extrapolation to humans of most of the other toxic effects noted in animals.

In humans, detection and examination of exfoliated bladder cells by urine cytology may be useful in detecting bladder cancer (Crosby et al. 1991; Mason et al. 1986; Tsuchiya et al. 1975). Although relevant

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to benzidine's principal toxicological effect of concern in humans, this biomarker would not be specific to benzidine exposure. It has been reported that bladder cancer patients who have been exposed to benzidine are at increased risk of developing multiple primary tumors at other sites in comparison with nonexposed bladder cancer patients (Morinaga et al. 1982; Okubo et al. 1985). This clinical presentation might suggest that a possible benzidine exposure has taken place.

Tanigawa et al. (1990) observed an unusual and persistent natural killer cell activity pattern toward a target human cancer cell line in individuals who had been exposed to benzidine or  $\beta$ -naphthylamine. Although overall activity was apparently normal, the unusual natural killer cell pattern was achieved by an increase in the number of circulating natural killer cells, each of which possessed a lower than normal per cell activity. This effect was observed even 20 years after exposure, and the mechanisms are currently unknown. Should this observation be confirmed and verified as a benzidine effect, investigation of its scientific basis, reproducibility, and specificity might allow for its development as a sensitive biomarker. This study also reported an increased proportion of Leu11a-(CD-16) positive circulating peripheral lymphocytes in workers exposed to benzidine or  $\beta$ -naphthylamine. A follow-up study (Araki et al. 1993) reported that the total and relative numbers of T lymphocyte subpopulations (CD4+ and CD3+) in the high-exposure group were significantly lower than those found in the control group. The authors concluded that measurement of CD4+ lymphocytes provides a useful biological marker of past exposure to aromatic amines, including benzidine. The more recent studies of Sung et al. (1995) and Tanigawa et al. (1996) also found changes in the numbers of T lymphocyte subpopulations in high-exposure groups of dye workers exposed to aromatic amines. Tanigawa et al. (1996) suggested that the observed changes in T lymphocytes resemble those noted in patients receiving immunosuppressive therapy.

Additional information concerning biomarkers for effects on the immune, renal, and hepatic systems can be found in the CDC/ATSDR Subcommittee Report on Biological Indicators of Organ Damage (CDC/ATSDR 1990), and on the neurological system in the Office of Technology Assessment Report on Identifying and Controlling Poisons of the Nervous System (OTA 1990). A more detailed discussion of the health effects caused by benzidine can be found in Section 3.2.

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**3.9 INTERACTIONS WITH OTHER CHEMICALS**

No specific quantitative data on the interaction of benzidine with other chemicals were located. However, it is reasonable to assume that the combined effect of the carcinogenic arylamines that impact the same target organs would be at least as great as the additive effect of their individual exposures. Data from at least one retrospective epidemiology study provide support for this hypothesis (Goldwater et al. 1965). It was reported that 21% of workers exposed to benzidine between 1912 and 1962 developed bladder cancer, while the rate was 46% for those exposed to both benzidine and another known bladder carcinogen,  $\beta$ -naphthylamine. The significance of these findings regarding a possible synergistic effect between the two chemicals cannot be evaluated because neither the levels of exposure nor the dose-response relationships could be adequately determined.

Rat studies on the chlorinated benzidine analog 3,3'-dichlorobenzidine suggest that concurrent or sequential exposure with other known bladder carcinogens results in at least an additive carcinogenic effect (Tatematsu et al. 1977; Tsuda et al. 1977). The relevance to benzidine of these findings is unclear because differences in metabolism between the two chemicals have been reported.

Though not universally found, synergistically elevated rates of bladder cancer in workers exposed to benzidine who also smoked were reported in several epidemiology studies (Bi et al. 1992; Wu 1988; Xue-Yun et al. 1990). These findings suggest interactions between benzidine and the carcinogenic or enzyme-inducing compounds found in cigarette smoke.

It is also possible that alcohol might potentiate the toxicity of benzidine, probably by altering its metabolism. Exposing rats to 15% (v/v) ethanol in drinking water for 6 weeks resulted in increased cytochrome P-450 content and enhanced N-acetylation capacity of isolated hepatocytes (Neis et al. 1985a). Further, relative to the mutagenicity mediated by control hepatocytes, the *in vitro* mutagenicity of benzidine to bacteria was increased following metabolic activation by hepatocytes isolated from the ethanol-treated rats. Again, the relevance of these findings to humans is unclear, given that the bladder appears to be the principal human target organ, and that noncytochrome P-450 pathways may be significant in the human metabolism of benzidine.

Finally, as discussed in Section 3.11.3, various pharmaceutical compounds may influence the toxicity of benzidine, either by affecting its metabolism or by interacting with its reactive metabolites.

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**3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE**

A susceptible population will exhibit a different or enhanced response to benzidine than will most persons exposed to the same level of benzidine in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of benzidine, or compromised function of organs affected by benzidine. Populations who are at greater risk due to their unusually high exposure to benzidine are discussed in Section 6.7, Populations With Potentially High Exposures.

Evidence suggesting that N-acetylation plays a key role in the metabolism and toxicity of benzidine has been previously addressed (Section 3.4.3), and the possibility of genetic polymorphism for acetylation capacity and cancer susceptibility to certain drugs and chemicals has recently been evaluated (Brown 1988). During the 1950s and 1960s, it was demonstrated that differential sensitivity to drugs such as isoniazid and sulfamethazine were based on a Mendelian distribution in the population of two genetic alleles for N-acetyltransferase, with the allele responsible for low acetyltransferase activity being recessive to that for high enzyme activity. In the United States, it has been estimated that approximately 50% of the population is homozygous for the slow allele, 40% heterozygous for the two alleles, and 8% homozygous for the fast allele (Brown 1988). This equates to phenotypic population of slow and fast acetylators (possibly also very fast acetylators—those homozygous for the fast allele). The relative proportions of these subpopulations can vary with ethnic origin and geographical location.

Recent studies have begun to elucidate the molecular basis of the human N-acetylation polymorphism through gene cloning and sequencing (Blum et al. 1991; Ohsako and Deguchi 1990). At least three mutant alleles with specific DNA base-substitutions have been isolated, and they appear to account for the vast majority of slow acetylator phenotypes. Two of the alleles seem to cause a decrease in amount of the NAT2 isozyme of N-acetyltransferase (probably by defective translation of the RNA message), whereas the third produces an unstable enzyme. Based on these findings, a simple DNA amplification assay was developed which appears to allow the predictive genotyping of more than 95% of the slow and fast acetylator alleles (Blum et al. 1991).

Since the early drug work, other chemicals, including benzidine, have been found to be differentially acetylated in humans (and rabbits), apparently by the same N-acetyltransferase involved in isoniazid and sulfamethazine metabolism (Glowinski et al. 1978; Peters et al. 1990). As suggested by the metabolism data previously discussed and as noted by Brown (1988), N-acetylation appears to contribute to

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benzidine's carcinogenic activation in the liver (e.g., rodents) but not in the bladder (e.g., rabbits, dogs), where it seems more likely to be involved in deactivation and elimination. Several studies in humans suggested that the slow acetylator phenotype significantly enhanced the risk of arylamine-induced bladder cancer (Cartwright 1983; Cartwright et al. 1982; Hanke and Krajewska 1990; Lower 1979; Lower et al. 1979; Mommsen and Aagaard 1986). However, more recent data indicate that the acetylation rate may not be as important as previously thought (Hayes et al. 1993; Lakshmi et al. 1995b; Rothman et al. 1996a; Zenser et al. 1996). For example, Lakshmi et al. (1995a) showed that human liver slices from individuals with the rapid NAT2 genotype formed only 1.4-fold more N-acetylbenzidine than slow acetylators; this difference was not significant. They also found no apparent correlation between NAT2 genotype and N-acetylbenzidine or N,N'-diacetylbenzidine formation. In experiments conducted with paraoxon, a deacetylase inhibitor, no difference in yield of N,N'-diacetylbenzidine was seen between slow and fast acetylators, suggesting that a second acetylation pathway may be involved in the human liver. Additional studies by the same group of investigators showed that both benzidine and N-acetylbenzidine are preferred substrates for NAT1 (Zenser et al. 1996). In a study of benzidine-exposed workers in India, almost all benzidine-related metabolites in the urine were acetylated among slow, as well as rapid acetylators, and NAT2 activity did not affect the levels of any DNA adduct measured (Rothman et al. 1996a). These findings suggested that it is unlikely that interindividual variation in NAT2 function is relevant for benzidine-associated bladder carcinogenesis (Rothman et al. 1996a). A study of 196 urothelial cancer patients in an area with earlier benzidine production in Germany also found no increased prevalence of slow acetylators in comparison with the general (unexposed) population (Golka et al. 1996) (see Section 3.5.2 for further discussion on acetylator type and bladder cancer risk).

In the same cohort evaluated by Rothman et al. (1996a), Rothman et al. (1997) evaluated the influence of urine pH on the proportion of urinary benzidine and N-acetylbenzidine present in the free, unconjugated state and on exfoliated urothelial cell DNA adducts. The authors found that post-workshift urine pH was inversely correlated with the proportions of benzidine and N-acetylbenzidine present as free compounds. They also noticed that the average of each subject's pre- and post-workshift urine pH was negatively associated with the predominant urothelial DNA adduct. These observations are consistent with *in vitro* studies of human (Babu et al. 1994a) and dog liver (Babu et al. 1992) preparations in which the glucuronides of benzidine, N-acetylbenzidine, and N'-hydroxy-N-acetylbenzidine became unstable (releasing a potential active intermediate) as urine pH was decreased from 7.4 to 5.5. The potential implication of this observation is that benzidine-exposed workers or individuals from the general population who smoke and consume foods that contribute to urine acidification (cheese, meat, fish, and grains) may be at a higher risk for bladder cancer.

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A deficiency of the glutathione S-transferase M1 (GSTM1) gene has been associated with bladder cancer in the general population in a number of studies (Bell et al. 1993; Brockmüller et al. 1996; Daly et al. 1993); however, other studies found no association between this gene deficiency and the incidence of bladder cancer (Lin et al. 1994; Zhong et al. 1993). In a recent case-control study (38 bladder cancer cases, 43 controls) of workers in China previously exposed to benzidine, the GSTM1-null genotype was not associated with increased risk of bladder cancer, although “highly exposed” workers with the null genotype exhibited a nonsignificantly greater risk of bladder cancer compared to similarly exposed workers without this allele (Rothman et al. 1996b). The GSTM1 genotype had no impact on urothelial cell DNA adduct and urinary mutagenicity levels in workers exposed to benzidine at the time of the study; additionally, human GSTM1 did not conjugate benzidine or its metabolites. Shinka et al. (1998) found no significant association between urothelial cancer patients and GSTM1 gene deficiency among 137 workers who had prior exposure to dyestuff intermediates, 36 of whom had urothelial cancer. However, in a multiple logistic analysis of the contributions of both GSTM1 gene polymorphism and occupational environmental factors, a “strong trend” toward development of urothelial cancer was noted.

As previously discussed, there is epidemiological evidence to suggest that people who smoke or are exposed to other arylamine carcinogens are likely to be at increased risk for benzidine-induced cancer (Goldwater et al. 1965). There is also laboratory evidence based on rats that indicates that significant alcohol consumption could increase susceptibility to benzidine toxicity (Neis et al. 1985a). Likewise, it is possible that populations having a diet high in meat and fat, and low in grain and fiber, might experience some excess risk due to resultant effects on benzidine metabolism by intestinal microflora (Gorbach and Goldin 1990; Levine 1991) (see Section 3.11.3).

#### **3.11 METHODS FOR REDUCING TOXIC EFFECTS**

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to benzidine. 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 benzidine. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

No texts were found that provided specific information about treatment following exposures to benzidine.



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**3.11.1 Reducing Peak Absorption Following Exposure**

No data were located regarding methods for reducing absorption after inhalation exposure to benzidine.

Following oral exposure, there are several methods by which the removal of benzidine from the gastrointestinal tract may be facilitated. Induced emesis, gastric lavage, use of saline cathartics, or activated charcoal are all common methods used to diminish the gastrointestinal absorption of compounds such as benzidine (Bronstein and Currence 1988; Sittig 1991; Stutz and Janusz 1988). In general, these treatments are most effective when used within a few hours after exposure. In some cases, these treatments may be contraindicated. For example, some authors contend that emesis should not be induced (Bronstein and Currence 1988), possibly due to risk of the chemical's aspiration into the lungs. In addition, emesis is definitely contraindicated in obtunded, comatose, or convulsing patients. Oils should not be used as a cathartic, as they may enhance the gastrointestinal absorption of benzidine. Saline cathartics should be used with caution in patients with impaired renal function.

Several methods exist for reducing absorption following dermal or ocular exposure to benzidine. All contaminated clothing should be removed, and contacted skin should be immediately washed with soap and water (Bronstein and Currence 1988; Sittig 1991; Stutz and Janusz 1988). Proparacaine hydrochloride may be used to facilitate eye irrigation (Bronstein and Currence 1988), which should be initiated immediately.

**3.11.2 Reducing Body Burden**

Activated charcoal is sometimes administered in serial doses to minimize the enterohepatic recirculation of persistent chemicals. Studies in humans and animals indicate that benzidine is cleared from the body fairly rapidly, most of which appears in the urine (Meigs et al. 1951, 1954). Although it has not been studied in humans, animal data suggest that enterohepatic circulation may be involved in the persistence and further metabolism of benzidine and its metabolites (Chipman and Mohn 1989). Relatively prompt administration of activated charcoal may, therefore, be of benefit.

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**3.11.3 Interfering with the Mechanism of Action for Toxic Effects**

Benzidine must undergo metabolic activation to produce its deleterious effects, probably through binding of oxidized reactive intermediates to nucleic acid (DNA, RNA) and protein target molecules. Currently, there is little that can be done to alter the progression of toxicological events once binding of reactive intermediates to target molecules has occurred. Therefore, interfering with the metabolic activation of benzidine and the activity of its reactive intermediates seems a more promising approach.

The only reports specifically addressing the interruption of benzidine metabolism focus on the prostaglandin H synthase activation pathway (Lakshmi et al. 1990b; Zenser and Davis 1990; Zenser et al. 1983), a pathway speculated to be of significance particularly for bladder and other nonhepatic carcinogenesis. These authors note that tissue level of peroxide is an important determinant of prostaglandin hydroperoxidase activity, and that catalase and glutathione peroxidase are the principal enzymes responsible for maintaining low levels of hydrogen peroxide and fatty acid hydroperoxides, respectively. Therefore, any actions capable of enhancing the activities of these enzymes might be of some value in inhibiting prostaglandin H synthase's cooxidation of benzidine. Tissue content of arachidonic acid, a substrate of prostaglandin H synthase, is normally very low, and steps to minimize its release from phospholipids after hormonal, neuronal, or mechanical stimuli would also be of theoretical value.

A specific inhibitor of the peroxidatic activity of prostaglandin H synthase is not currently available, but thioureyline drugs (propylthiouracil, methimazole) have been used to competitively inhibit the activity of thyroid peroxidase. Prostaglandin H synthase, however, is capable of generating its own peroxide substrate through its cyclooxygenase activity. Inhibition of this cyclooxygenase by nonsteroidal anti-inflammatory drugs such as aspirin can result in reduced prostaglandin hydroperoxidase activity, if the endogenous tissue content of peroxide is sufficiently low. In this respect, supplemental treatment with antioxidants might prove beneficial.

Zenser and his colleagues (Zenser and Davis 1990; Zenser et al. 1983) further propose the following model of benzidine activation in which prostaglandin H synthase activity itself may be inhibited at any one of several steps. First, generation of peroxide cosubstrate by the enzyme's own cyclooxygenase could be inhibited with aspirin or indomethacin. In some cases, peroxide formation from sources other than fatty acids might also be minimized (i.e., inhibition of lipoxygenase activity with phenidone under conditions of high peroxide tone).

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Second, the actual prostaglandin hydroperoxidase-catalyzed activation of benzidine would likely be interrupted by competitive substrates for the enzyme. These might also compete with benzidine for possible interaction with other enzyme-generated oxidants which have been proposed to oxidize cosubstrates such as benzidine by chemical reaction. Such competitive inhibitors include propylthiouracil, phenidone, methimazole, and MK447.

Third, the oxidized reactive intermediates (e.g., benzidinediimine) could be reduced back to the parent compounds (e.g., benzidine) by reducing agents such as ascorbic acid (vitamin C). Antioxidants (such as ethoxyquin and butylated hydroxytoluene) have been shown to inhibit the carcinogenicity of a number of compounds presumed to be activated by the cytochrome P-450 system, but may also be postulated to act at either the second or third steps just noted in the proposed prostaglandin H synthase pathway.

Finally, benzidine and its oxidized reactive intermediate, benzidinediimine, may be inactivated by conjugation with molecules such as glutathione. Zenser et al. (1983) point out that only intervention via this latter step results in the actual elimination of benzidine, whereas steps 1–3 simply prevent the formation of, or reduce the amount of, already existing activated intermediates. Also, the inhibition of the fatty acid cyclooxygenase activity in step 1 has the potential disadvantage of altering physiologically important prostaglandin and thromboxane synthesis.

Evidence supporting the possible efficacy of such intervention was found in a study by Zenser et al. (1983). When expressed as a percentage of control values, the levels of benzidine's prostaglandin H synthase-catalyzed *in vitro* binding to microsomal protein were significantly ( $p < 0.05$ ) lower after treatment with most of the above compounds in the presence of cosubstrate (arachidonic acid/hydrogen peroxide): propylthiouracil (51/30%), methimazole (27/17%), MK447 (44/38%), phenidone (6/5%), indomethacin (<1/122%), glutathione (26/12%), and vitamin C (50% at 0.05 mmol using arachidonic acid, or 1% at 1 mmol using hydrogen peroxide). Only indomethacin in the presence of hydrogen peroxide cosubstrate was found ineffective. Similarly, several of the compounds were demonstrated to substantially reduce the prostaglandin H synthase-mediated *in vitro* binding of benzidine to DNA in the presence of arachidonic acid; values expressed as a percentage of the control were: glutathione (4%), vitamin C (<1%), aspirin (4%), and indomethacin (9%).

It therefore seems possible that pharmacological intervention following benzidine exposure might be of value in mitigating deleterious effects, especially as the antioxidant, reducing, and conjugating types of

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compounds discussed above would be expected to also be of value for metabolic activation catalyzed by other enzymes (cytochrome P-450, flavin monooxygenases, and lipoxygenases).

As discussed in a number of review articles (e.g., Gorbach and Goldin 1990; Levine 1991), and as suggested by some of the bacterial acetyltransferase studies referred to previously (Section 3.4.3), microflora of the gastrointestinal tract may also play a significant role in the metabolic activation of benzidine. Microbial enzymes relevant to the generation of benzidine from certain azo dyes, or to its subsequent metabolism, include azoreductases,  $\beta$ -glucuronidases, and acetyltransferases. It is possible that a diet high in grain and fiber and low in meat and fat could contribute to lower risk following benzidine or benzidine-based azo dye exposure by lowering levels of the first two types of enzymes (Gorbach and Goldin 1990).

#### 3.12 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 benzidine 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 benzidine.

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.

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**3.12.1 Existing Information on Health Effects of Benzidine**

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to benzidine are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of benzidine. 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 Identifying 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.

As shown in Figure 3-6, no human data exist on the lethality of benzidine (other than death by cancer) for any route of exposure. With the exception of two studies on the acute and chronic effects of dermal exposure, no information was located regarding the systemic effects in humans of benzidine exposure by any route or for any duration. Four studies were located regarding immunologic effects and a study of genotoxic effects in occupational settings where inhalation is assumed to be the main route of exposure. A single study addressed developmental effects and possible environmental (principally oral) exposure to benzidine. Otherwise, no information was located regarding neurologic or reproductive effects after any route of exposure, or regarding developmental effects after inhalation or dermal exposure. A single study addressed developmental and cancer effects in residents living near a benzidine-contaminated Superfund site, where the principal route of exposure is presumed to have been oral. Many epidemiological studies were located regarding cancer effects, all of which explicitly or implicitly involved inhalation and/or dermal exposure.

For animals, no information was found regarding any toxic effect after dermal exposure. With the exception of a single rat cancer study, there was a similar lack of data regarding toxic effects after inhalation exposure. For the oral route of exposure, data were located regarding death, intermediate- and chronic-duration systemic effects, and immunologic, neurologic, genotoxic, and carcinogenic effects in animals. However, no information was found regarding acute-duration systemic effects or developmental effects in animals after oral exposure to benzidine.

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**Figure 3-6. Existing Information on Health Effects of Benzidine**

	Systemic									
	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
Inhalation				•				•	•	
Oral							•		•	
Dermal		•	•							•

**Human**

	Systemic									
	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
Inhalation										•
Oral	•		•	•	•	•			•	•
Dermal										

**Animal**

- Existing Studies

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**3.12.2 Identification of Data Needs**

**Acute-Duration Exposure.** Only one study was located regarding the acute systemic effects of benzidine in humans. Severe recurrent eczematous dermatitis was reported in one individual following dermal exposure to benzidine in a clinical laboratory setting (Baer 1945). In animals, oral LD<sub>50</sub> values have been reported for rats and mice (DOT 1972). Very early work provides some data as to the oral doses which can lead to death in rabbits and dogs (Adler 1908), but these studies lack experimental detail and information on benzidine purity and chemical form. Potential immunotoxicity was indicated in one recent acute-duration oral mouse study where exposure to a nonspecified dose of benzidine was reported to cause a delayed hypersensitivity response, altered lymphocyte blastogenesis and natural killer cell activity, and an altered spleen/body weight ratio (Luster et al. 1992). No other acute studies in animals were located. The information with respect to both target organ identification and levels of exposure necessary to cause acute toxic effects is insufficient to derive acute-duration inhalation or oral MRLs. Because exposure to benzidine at hazardous waste sites is most likely to be by ingestion, emphasis should be placed on studies that examine effects following oral exposure, preferably studies that could be utilized to derive an acute oral MRL for benzidine. While an acute inhalation MRL for benzidine is also lacking, additional inhalation studies are not considered a priority as exposure at hazardous waste sites is less likely to be by the inhalation route. Likewise, additional acute dermal studies are not considered a priority due to the unlikelihood of significant dermal exposures to benzidine at hazardous waste sites as well as the predicted relatively poor bioavailability from soil based on its strong attachment to soil. Pharmacokinetics data are available in various animal species as well as in humans.

**Intermediate-Duration Exposure.** No studies were located regarding systemic effects in humans after inhalation, oral, or dermal exposure to benzidine. In animals, there are some oral intermediate-duration data which describe cardiovascular, hepatic, and renal (DePass and Morris, 1981; Oida 1958a, 1958b), hematological (Nelson et al. 1982), immunological (Rao et al. 1971), or weight-loss effects (DePass and Morris 1981; Schieferstein 1982). However, these data are too limited to allow for the identification of the critical target organs or the NOAEL or LOAEL values for intermediate-duration oral exposure, and therefore, are inadequate for derivation of an oral MRL. Data for the inhalation and dermal routes of exposure were not located. Additional oral intermediate-duration studies that identify target organs and provide quantitative data on the threshold for specific toxic effects are needed in mice or rats, rabbits, and dogs. Toxicokinetic and cancer data are known for these species and for humans, which allows for easier extrapolation of any observed intermediate-duration effects to humans. Oral exposure via contaminated media is thought to be the potential main route of exposure to benzidine at or near waste

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sites. Therefore, additional oral studies in animals involving a range of exposure concentrations and employing sensitive histological and biochemical measurements of injury to a comprehensive set of organs or systems are needed to establish dose-response relationships and identify thresholds for these effects. Special emphasis should be placed on reproductive organ pathology since, as discussed below, information on reproductive effects is lacking. Although ATSDR considers skin contact with contaminated soil a source of potential exposure to benzidine at hazardous waste sites, at this time, only oral studies are given priority consideration. This is because no experimental data were located regarding the bioavailability of benzidine attached to soil particles. However, benzidine is a weak base that can be irreversibly bound to soil matrices, thus limiting its potential bioavailability from soil surfaces. Inhalation data needs are not assigned priority because inhalation is not considered to be a primary exposure route at hazardous waste sites. No intermediate-duration pharmacokinetics data were located, but a need for such data is not apparent at this time.

**Chronic-Duration Exposure and Cancer.** No studies were located regarding systemic effects in humans after inhalation or oral exposure to benzidine for chronic durations. Dermal sensitization to benzidine was detected in 231 (5%) of 4,600 patients tested over a 5-year period; of these, 208 (89%) were clinically diagnosed as suffering from occupational contact dermatitis (Grimalt and Romaguera 1981). No studies were located regarding systemic effects in animals after chronic inhalation or dermal exposure to benzidine, so no inhalation MRLs could be calculated. Several studies provided data on chronic-duration oral effects in animals, but, as detailed below, were considered inadequate for derivation of a chronic oral MRL. Brain vacuolization, a serious effect, was seen in mice exposed to approximately 1.8 mg/kg/day, the lowest dose tested in the Littlefield et al. (1983) study. Weight loss (Schieferstein 1982), liver foci—which may represent preneoplastic lesions (Frith et al. 1980), hemosiderin pigmentation of the spleen, and bile duct hyperplasia (Littlefield et al. 1983) have been reported in mice after chronic-duration oral exposure to benzidine doses between approximately 2.5 and 11 mg/kg/day. A marginally significant ( $p < 0.10$ ) increase in proteinaceous kidney casts in female mice was reported, but not the specific dose at which the effect was observed (Nelson et al. 1982). Liver cirrhosis was observed in several rabbits orally exposed for up to 3.5 years (Bonser 1959); however, only a summary of the data was presented. Recurring renal cystitis was the only treatment-related systemic effect observed in seven dogs exposed for 5 years (Spitz et al. 1950), but incomplete reporting of the results precluded establishing with certainty the dose level at which this effect occurred. Future epidemiological studies of exposed workers investigating such noncancer end points rather than focusing only on cancer are needed, as are additional noncancer chronic-duration studies in animals, although data from a 90-day oral study should be evaluated before conducting chronic toxicity studies. These studies are important because



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humans may be exposed to benzidine for chronic-durations near hazardous waste sites; such studies might be useful for the development of a chronic oral MRL. Oral data would be most relevant with respect to the most likely human exposure scenarios. Chronic-duration pharmacokinetics data were not located, but the need for such studies is not apparent at this time.

Epidemiological evidence is currently sufficient to qualitatively establish that benzidine is a human bladder carcinogen following occupational exposure (Bi et al. 1992; Bulbulyan et al. 1995; Ferber et al. 1976; Goldwater et al. 1965; Mancuso and El-Attar 1967; Mason et al. 1986; Meigs et al. 1986; Montanaro et al. 1997; Naito et al. 1995; Shinka et al. 1991, 1995; Tsuchiya et al. 1975; Vigliani and Barsotti 1962; Zavon et al. 1973). Some studies have observed benzidine-associated increased risks for cancer at one or more other human tissue sites as well (i.e., stomach, kidney, central nervous system, oral cavity, larynx, esophagus, liver, gallbladder, bile duct, and pancreas) (Bulbulyan et al. 1995; Delzell et al. 1989; Montanaro et al. 1997; Morinaga et al. 1982; Morikawa et al. 1997; Okubo et al. 1985; Piolatto et al. 1991; Wu 1988). Confirmation of these latter findings would be worthwhile, especially in the context of species and organ differences in benzidine metabolism and susceptibility to cancer. The exposure pathways in the workplace are not known with certainty, but most probably involve a mixture of inhalation and dermal routes. One study was located regarding cancer in humans after potential oral exposure to benzidine (Budnick et al. 1984). In this study, elevated rates of cancer of the bladder and several other sites were detected in residents living near a Superfund site contaminated with benzidine and other contaminants. Benzidine could not specifically be identified as a causative agent. Additionally, an elevated risk of bladder cancer for Japanese kimono painters who licked benzidine-based dyes from their utensils has been reported (Yoshida 1971; Yoshida and Miyakawa 1973). A study (Hanke and Krajewska 1990) has confirmed the observation that a preponderance (88% in this study) of occupationally-exposed bladder cancer patients possess the slow acetylator phenotype, thus reaffirming this characteristic as a human risk factor for developing cancer of the bladder. However, more recent data indicate that the acetylation rate may not be an important risk factor for developing bladder cancer (Hayes et al. 1993; Rothman et al. 1996a; Zenser et al. 1996). A study using cluster analysis to examine groups of jobs with similar exposures to certain chemicals has identified an apparent relationship between exposure of parents to arylamines (including benzidine) during preconception, prenatal, or postnatal periods and the occurrence of brain tumors in their children (Wilkins and Sinks 1990). When analysis was focused on inferred exposure to benzidine, exposure of parents during the postnatal period was associated with an even greater excess risk for brain cancer in their children. However, these observations were not statistically significant, and parts of the analytical methodology have not yet been verified.

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In animals, myeloid leukemia, several mammary tumors, and one hepatoma were reported in 28 rats exposed to benzidine by inhalation (Zabezhinskii 1970). A new inhalation study using more animals and a standardized protocol would be useful in expanding and extending these observations. A 30-day oral exposure was reported to induce mammary carcinoma in female rats (Griswold et al. 1968). A lifetime exposure study on both sexes with complete histopathology would be helpful in confirming and expanding this limited study. Hamsters fed benzidine base or benzidine dihydrochloride developed liver and bile duct tumors, but no bladder pathology was observed (Saffiotti et al. 1967). The primary cancer site in mice after oral exposure to benzidine is the liver (Frith et al. 1979, 1980; Littlefield et al. 1983, 1984; Nelson et al. 1982; Schieferstein 1982), but Harderian gland, uterine, and lung tumors, as well as reticulum cell sarcomas have also been observed (Littlefield et al. 1983, 1984). Older, relatively limited studies have indicated that rabbits may be relatively insensitive to bladder cancer induction after oral exposure to benzidine. Of 13 rabbits exposed, 1 rabbit developed bladder carcinoma and another developed gallbladder adenocarcinoma (Bonser 1959). No tumorigenesis at any site was noted in another study where rabbits were exposed for 20–128 days (Oida 1958a, 1958b). Oral exposure to benzidine has been shown to induce bladder cancer in dogs (Spitz et al. 1950). Exposure by subcutaneous injection has provided some additional carcinogenicity data for rats (liver and some colon tumors) (Spitz et al. 1950). Intraperitoneal injections of benzidine also increased the incidences of mammary and Zymbal's gland tumors in rats (Morton et al. 1981). No studies were located regarding cancer in animals after dermal exposure to benzidine.

Cancer, particularly bladder cancer, should continue to be evaluated in future epidemiological studies of occupationally exposed workers and in populations living near waste sites where benzidine has been found. However, there is no need for further animal studies at this time. Screening studies are currently being conducted in a cohort of workers occupationally exposed to benzidine both to develop a bladder cancer screening approach and to reduce the number of deaths and serious morbidity associated with bladder cancer in this cohort (see Section 3.12.3).

**Genotoxicity.** Exposure (presumed to be primarily by inhalation) to benzidine and benzidine-based dyes has recently been shown to induce chromosomal aberrations and polyploidy in the circulating peripheral lymphocytes of workers (Mirkova and Lalchev 1990). These observations provide evidence of benzidine's genotoxic potential in humans in an occupational-exposure setting, and are supported by positive gene mutation data in *D. melanogaster* (Fahmy and Fahmy 1977; Graf et al. 1989), as well as by positive results in rodents following oral or intraperitoneal injection exposure for micronucleus induction (Cihak 1979; Mirkova 1990; Mirkova and Ashby 1988; Sanderson and Clark 1993; Tice et al. 1990;

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Urwin et al. 1976), chromosomal aberration induction (Das et al. 1994; Sinsheimer et al. 1992; Talaska et al. 1987), DNA adduct formation (Phillips et al. 1990), DNA damage (Parodi et al. 1981; Petzold and Swenberg 1978), and unscheduled (repair) DNA synthesis (Ashby and Mohammad 1988; Ashby et al. 1990) (see Table 3-3). When tested in many *in vitro* assays (Table 3-4), benzidine has generally tested positive for reverse mutation in *Salmonella typhimurium* in the presence of exogenous metabolic activation (e.g., liver S-9) (Ames et al. 1973; Chung et al. 2000; Dorado and Pueyo 1988; Duverger-van Bogaert et al. 1995; Gregory et al. 1981; Zeiger et al. 1992); negative for SOS DNA repair in *Escherichia coli* (Von der Hude et al. 1988); positive for mutation in yeast (Buchholz et al. 1992; Mitchell and Gilbert 1991); positive (Oberly et al. 1990) or negative (Phillips et al. 1990) for gene mutation in Chinese hamster ovary cells; positive (Fassina et al. 1990; Suter et al. 1992) or negative (O'Donovan 1990; Oglesby et al. 1983) for gene mutation in Chinese hamster V79 cells; and positive (TK locus) or negative (HGPRT locus) for gene mutation in mouse lymphoma cells (Henderson et al. 1990; Myhr and Caspary 1988). Benzidine has also tested positive for chromosome breaks (Swenberg et al. 1976) and sister chromatid exchange (Grady et al. 1986; Lindahl-Kiessling et al. 1989) in cultured human and animal cells; generally positive in cultured hepatocytes for unscheduled DNA synthesis (Kornburst and Barfknecht 1984a, 1984b; Steinmetz et al. 1988; Williams 1978); positive for animal cell transformation (Ashby et al. 1978; Pienta 1980); and negative in cultured mammalian cells (but positive with calf thymus DNA) in the absence of exogenous activation for DNA adduct formation (Phillips et al. 1990) (see Table 3-4).

**Reproductive Toxicity.** No studies were located on reproductive effects in humans after exposure to benzidine by any route, for any duration. Very limited information was found regarding reproductive effects in animals. Increased incidence of atrophy of the ovaries was reported in mice receiving approximately 7.2 mg benzidine/kg/day in the drinking water in a chronic study (Littlefield et al. 1983). In a study in which mice were intraperitoneally injected once per day for 5 days with 27.5–440 mg/kg/day of benzidine, no adverse effects on sperm morphology were observed (Wyrobek et al. 1981). This limited information is insufficient for drawing conclusions regarding the possible reproductive effects of benzidine in humans. The oral route of exposure is considered the primary route of exposure to benzidine at or near waste sites. There is no evidence of adverse reproductive effects in workers that have been exposed to benzidine. Since there is practically no information on reproductive effects of benzidine in humans or in animals, it is recommended that reproductive organ pathology be evaluated in the 90-day oral study previously identified as data need. Multi-generation animal studies do not seem warranted at this time. Inhalation and dermal data needs are not assigned priority because they are not primary exposure routes at hazardous waste sites.

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**Developmental Toxicity.** A retrospective epidemiology study examined the incidence of birth defects in residents living near a Superfund site contaminated with benzidine,  $\beta$ -naphthylamine, and benzene (Budnick et al. 1984). The actual extent of exposure (if any) to benzidine was not determined. If any exposure occurred, it was most probably oral, but other routes and/or toxicants could also have been involved. No increased incidence was noted for birth defects in general, nor for any specific birth defect. A study by Wilkins and Sinks (1990) suggested an association between parental exposure to benzidine and childhood brain tumors. However, results and conclusions of this study are largely speculative due to a lack of sufficient validation of the job exposure matrix used to estimate exposure levels. There is no evidence that offspring from workers exposed to benzidine have experienced adverse developmental effects. No studies were located on developmental effects in animals after exposure to benzidine by any relevant route. However, a study in which increased frequency of micronucleated polychromatic erythrocytes was observed in the liver of mice fetuses from dams injected intraperitoneally with benzidine suggests that benzidine (or metabolites) can cross the placenta (Sanderson and Clark 1993). The limited information available is insufficient to draw conclusions regarding the possible developmental effects of benzidine in humans. The oral route of exposure is considered the primary exposure route to benzidine at or near waste sites. Given the limitations of the anecdotal report of Wilkins and Sinks (1990) and the limited evidence in animals that benzidine (or metabolites) crosses the placenta after intraperitoneal injection (Sanderson and Clark 1993), it is recommended that the possibility of placental transfer of benzidine or metabolites be first confirmed in an oral exposure study. Should placental transfer be established, pilot animal studies that provide information on the potential embryotoxicity, fetotoxicity, and teratogenicity of benzidine may be considered. Structure activity relationship analyses performed using the TOPKAT software showed benzidine to be a developmental non-toxicant (ATSDR 1999). Inhalation and dermal data needs are not assigned priority because they are not primary exposure routes at hazardous waste sites.

**Immunotoxicity.** Recent studies of workers exposed to benzidine (and simultaneously to other amines as well) have reported a decrease (per cell) of natural killer activity against a target human cancer cell line (Tanigawa et al. 1990) and changes in numbers of some T lymphocyte populations (Araki et al. 1993; Sung et al. 1995; Tanigawa et al. 1996). Although the status of the immune system was apparently not compromised by these effects, continued evaluation of these workers, and of other cohorts that might be identified, could be of value in an attempt to confirm these findings and to look for additional evidence of immunologic alterations. Should the effect be substantiated, it would be of interest to investigate the underlying mechanisms, especially because in some cases, the effect was noted nearly 20 years after the last arylamine exposure. One 5-day oral mouse study has identified several manifestations of

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immunotoxicity, including altered natural killer cell activity (Luster et al. 1992); however, doses causing the effects were not specified. Thus, additional quantitative animal studies to help define these apparent immunotoxic effects, their mechanisms, and associated dose-response relationships would be valuable. This could be accomplished in a 90-day oral study that examines lymphoid tissues and relevant blood components (i.e., peripheral lymphocytes). No inhalation or dermal studies are needed at this time because they are not primary exposure routes at hazardous waste sites.

**Neurotoxicity.** No studies were located regarding neurotoxic effects in humans after inhalation, oral, or dermal exposure to benzidine for any duration. In animals, a significant incidence of spongiform leukoencephalopathy (vacuolization of brain cells) was observed at the lowest dose tested (1.8 mg/kg/day) in a large, oral lifetime study in mice (Littlefield et al. 1983; Morgan et al. 1981). The effect was dose-dependent and specific to a certain region of the brain. The relevance of this finding to human health is unknown. The fact that no studies were located that reported neurological effects among workers exposed to benzidine suggest that the nervous system may not be a target for benzidine toxicity. No studies were located regarding neurological effects in animals after inhalation or dermal exposure. Considering the findings of Littlefield et al. (1983) and Morgan et al. (1981), it may be valuable to evaluate neuropathology in a 90-day study by the oral route, the primary route of exposure for populations living near waste sites. The inhalation and dermal route of exposures are not considered primary routes of exposure at hazardous waste sites.

**Epidemiological and Human Dosimetry Studies.** With the exception of those who may live near certain hazardous waste sites or a small number of factories, the probability of exposure of the general population to benzidine is very low. Benzidine is no longer produced for commercial sale in the United States. There are a number of published epidemiology studies that provide evidence that benzidine is a carcinogen in humans, but these studies do not provide good quantitative exposure data and focus mainly on bladder cancer (Bi et al. 1992; Bulbulyan et al. 1995; Ferber et al. 1976; Goldwater et al. 1965; Mancusco and El-Attar 1967; Mason et al. 1986; Meigs et al. 1986; Montanaro et al. 1997; Naito et al. 1995; Shinka et al. 1991, 1995; Tsuchiya et al. 1975; Vigliani and Barsotti 1962; Xue-Yun et al. 1990; Zavon et al. 1973). Some epidemiological studies have also suggested an association between benzidine exposure and risk for cancer at one or more tissue sites as well (Bulbulyan et al. 1995; Delzell et al. 1989; Montanaro et al. 1997; Morikawa et al. 1997; Morinaga et al. 1982; Okubo et al. 1985; Piolatto et al. 1991; Wu 1988). Thus, there remains a need for epidemiological data that can provide quantitative human dose-response information, and that can better address end points such as cancer at other sites and alterations of immunocompetence. The controversy of whether slow acetylators are at higher risk for

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benzidine-induced bladder cancer than fast acetylators appear to have been answered (Rothman et al. 1996a; Zenser et al. 1996) such that further research on this issue seems of questionable value at this time. In addition to cancer, studies of workers exposed to benzidine (and simultaneously to other amines) have reported a decrease (per cell) of natural killer activity against a target human cancer cell line (Tanigawa et al. 1990) and changes in numbers of some T lymphocyte populations (Araki et al. 1993; Sung et al. 1995; Tanigawa et al. 1996). It should be mentioned, however, that the status of the immune system was apparently not compromised by these effects. With the exception of those who may live near certain hazardous waste sites or a small number of factories, the probability of exposure of the general population to benzidine is very low. Benzidine is no longer commercially produced or used in the United States and it has been detected in only 28 of 1,585 NPL hazardous waste sites. However, in the unlikely event that exposure of the general population (in the past or present) primarily to benzidine is identified, individuals should be monitored for bladder cancer incidence and immunological effects.

**Biomarkers of Exposure and Effect.**

**Exposure.** A variety of benzidine-related compounds can be detected in the urine of humans exposed to benzidine or benzidine-based dyes (DeMarini et al. 1997; Engelbertz and Babel 1953; Meigs et al. 1951, 1954; NIOSH 1980c; Rothman et al. 1996a; Sciarini and Meigs 1961; Troll et al. 1963). These include benzidine itself, hydroxylated and or acetylated metabolites, and various glucuronide, glutathione, and sulfate conjugates. High performance liquid chromatography coupled with absorbance detection was reported to permit identification of urinary concentrations of benzidine and its monoacetylated derivative as low as 0.25 µg/L and of its diacetylated derivative as low as 0.5 µg/L (Dewan et al. 1988). Urinary detection of benzidine and its metabolites is suitable only for recent exposure scenarios, as animal studies indicate that most excretion occurs within the first week or so following exposure (Kellner et al. 1973; Lynn et al. 1984; Shah and Guthrie 1983). In studies with rats, hemoglobin adducts of benzidine and some of its metabolites have been detected by high performance liquid chromatography coupled with an electrochemical detector (Birner et al. 1990; Neumann 1988; Zwirner-Baier and Neumann 1998). This may prove a useful biomarker for exposures of intermediate-duration (or for detection of exposure after such intervals have elapsed since the last exposure), as the data suggest that levels of hemoglobin adducts are proportional to dose over a large dose range, and are indicative of cumulative exposure during the lifetime of the erythrocytes (120 days in humans). There are biomarkers of long-term exposure to benzidine. DNA adducts of benzidine and its metabolites can also be extremely sensitive biomarkers for detecting and quantifying exposure. Such adducts could be detected in as little as 1 µg of mouse hepatic DNA, after as little as 3 hours of exposure (Levy and Weber 1988). Similarly attomole ( $10^{-18}$  mole)

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quantities of benzidine-DNA adduct could be detected in human peripheral lymphocytes exposed *in vitro* to benzidine (Gupta et al. 1988). DNA adducts have also been detected in peripheral white blood cells (Zhou et al. 1997) and urine (Rothman et al. 1996a, 1997) from workers exposed to benzidine. Analysis of mutations in specific genes in bladder cancer cases will continue to provide important information for the characterization of bladder cancer induced by benzidine and other arylamines (Novara et al. 1996; Sorlie et al. 1998; Yasunaga et al. 1997). These procedures might help in medical surveillance for early detection of cancer and treatment.

**Effect.** It appears likely that most of benzidine's toxic effects are mediated through the binding of its activated metabolites to cellular macromolecules. Therefore, the previously discussed benzidine-derived adducts with DNA and hemoglobin may also be considered biomarkers of effect which could eventually be used for dosimetry. Experiments with radiolabeled benzidine or N-acetylbenzidine have demonstrated significant amounts of binding to DNA in the liver, kidney, and bladder of dogs (Beland et al. 1983; Lakshmi et al. 1990a). The highest concentration of binding was found in the bladder transitional epithelium, the principal site of benzidine's carcinogenicity in this species. Elevated frequencies of chromosomal aberrations and polyploidy have recently been detected in the circulating peripheral lymphocytes of workers exposed to benzidine and benzidine-based dyes (Mirkova and Lalchev 1990), and bacterial mutagenicity has been detected in the urine and bile of rodents treated with benzidine (Bos et al. 1980, 1981; Chipman and Mohn 1989). However, these and other manifestations of benzidine's genotoxicity are not specific to benzidine or even to arylamines. Reduced arylsulfotransferase activity in liver cytosol was reported in rats exposed to benzidine, and may be characteristic of many rat hepatocarcinogens (Ringer and Norton 1988). Its sensitivity, relative specificity, and relevance as a potential human biomarker are not currently known. The use of urinary cytology to detect and examine exfoliated bladder cells has been reported to be useful in screening for human bladder cancer (e.g., Crosby et al. 1991; Mason et al. 1986; Tsuchiya et al. 1975). By itself, this biomarker is not specific to benzidine exposure, but studies investigating the possible detection of benzidine-DNA adducts in such cells may be of value in enhancing specificity. Several reports observed subtle immunologic alterations in workers exposed to benzidine and/or  $\beta$ -naphthylamine (Araki et al. 1993; Sung et al. 1995; Tanigawa et al. 1990, 1996). Confirmation of these findings and an investigation of its basis, reproducibility, and agent-specificity could be useful in determining whether this effect has any potential as a relatively easily monitored biomarker. Evaluation of additional possible biomarkers will in part depend on the identification of additional toxic effects from any further acute-, intermediate-, or chronic-duration studies.

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**Absorption, Distribution, Metabolism, and Excretion.** No quantitative studies were located regarding the rate or extent of absorption in humans or animals after inhalation or oral exposure to benzidine. However, relatively rapid absorption can be inferred from an occupational study in which urinary levels of benzidine increased 4-fold during a workshift involving primarily inhalation exposure (Zavon et al. 1973). Similarly, significant absorption of benzidine-based dyes was indicated by the detection of benzidine and its acetylated metabolites in the urine of workers exposed to the dust of Direct Black 38 (Dewan et al. 1988). However, the relative contributions of pulmonary, oral, and dermal absorption were not addressed. Absorption after oral exposure to benzidine in mice (Martin et al. 1982) and dogs (Beland et al. 1983) was indirectly indicated by covalent binding of benzidine metabolites to DNA in liver and bladder tissue. Benzidine produced by microfloral reduction of benzidine-based dyes in the intestines was indirectly determined to be absorbable, and benzidine (but not the dyes) was demonstrated to be transported across the mucosa of an isolated segment of rat intestine in a perfusion chamber (Bos et al. 1986). Benzidine, in combination with acetone or DMSO, sodium lauryl sulfate, and/or methyl nicotinate was shown to be absorbed by isolated perfused porcine skin flaps; the addition of the reducing agent  $\text{SnCl}_2$  resulted in an inhibition of benzidine absorption (Baynes et al. 1996). No quantitative studies were located regarding absorption in humans after dermal exposure to benzidine, but benzidine and its metabolites were detected in the post-workshift urine of workers exposed to benzidine (Ferber et al. 1976; Meigs et al. 1951, 1954; Sciarini and Meigs 1961). After applying radiolabeled benzidine to the shaved skins of rats, the percentages of applied radioactivity detected in the blood were 0.2, 0.3, and 0.7% after 1, 8, and 24 hours, respectively (Shah and Guthrie 1983). Benzidine-derived radioactivity was distributed throughout the body, with approximately half still remaining at the site of application after 24 hours. A study of dermal application of radiolabeled benzidine to the shaved skin of rabbits and rats reported 3 and 5% of the radioactivity detected in the urine and feces of the rabbits, respectively, and 0.05 and 0.16% in the urine and feces of the rats, respectively (Aldrich et al. 1986). Although sparse, the collective data suggest that benzidine can be absorbed by all three major routes, with absorption and distribution occurring fairly rapidly after inhalation or oral exposure. The relative contribution of inhalation, oral, and dermal routes to typical occupational exposures remains one area where further studies could be useful. More recent studies have also provided evidence of inhalation absorption in workers exposed to benzidine (DeMarini et al. 1997; Rothman et al. 1996a).

No studies were located regarding distribution in humans after inhalation, oral, or dermal exposure to benzidine.



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No studies were located regarding distribution in animals after inhalation or oral exposure to benzidine or benzidine-based dyes. At 1, 8, and 24 hours after application of radiolabeled benzidine to the skin of rats (Shah and Guthrie 1983), organ distribution of radioactivity was approximately as follows: liver (1.5, 1.0, and 0.7%), lung (0.09, 0.2, and 0.2%), intestines (1.0, 14.0, and 1.3%), stomach (0.5, 0.4, and 0.08%), and carcass (1.9, 4.1, and 6.9%). After 24 hours, approximately half of the radioactivity remained at the site of application, while 41% was found in the urine and feces. Several studies have reported distribution data for time points ranging from less than 1 hour to 3–7 days after intravenous administration of benzidine to rats, dogs, and monkeys (Kellner et al. 1973; Lakshmi et al. 1990a; Lynn et al. 1984). It appears that benzidine clearance from the blood is at least several fold more rapid than that of its metabolites, that clearance is biphasic with most (90%) occurring during the first 24 hours, and that radiolabel is distributed throughout much of the body, relative amounts often reflecting species specificity for cancer target organs. Data from rats and mice also suggest that enterohepatic recycling may contribute to the continued retention, further metabolism, and toxicity of benzidine and its metabolites (Chipman and Mohn 1989). Although the contribution of the inhalation, oral, and dermal routes to benzidine body burden has not been clearly established in occupational studies, the mechanism of benzidine toxicity is not route-related, and therefore, additional animal studies to examine distribution of benzidine do not appear necessary at this time.

There is substantial information available from human, animal, and *in vitro* studies concerning various aspects of benzidine's complex metabolism. Numerous *in vivo* metabolites in a variety of species have been reported (EPA 1980a, 1986c; Whysner et al. 1996; Zenser et al. 1998). The urine and bile of humans and animals have been reported to contain free benzidine, a variety of hydroxylated and/or acetylated metabolites, and a number of metabolite conjugates with glucuronide, glutathione, and sulfate (EPA 1980a, 1986c; Lynn et al. 1984; Sciarini and Meigs 1958, 1961). Although no studies were located to demonstrate that metabolism may be substantially influenced by route of exposure, determining the relative importance of several potential metabolic pathways in hepatic versus nonhepatic tissue could lead to a better understanding of the mechanisms of benzidine toxicity and carcinogenicity. As with many other arylamines, benzidine is thought to require metabolic activation through N-oxidation to electrophilic derivatives capable of binding DNA in order to manifest its genotoxicity and carcinogenicity (Beland et al. 1983; Brouns et al. 1982; Iba 1987). Studies have shown that three main chemical reactions are involved in the metabolism of benzidine: N-acetylation, N-oxidation, and N-glucuronidation (Babu et al. 1992, 1993a, 1994a, 1994b, 1995; Frederick et al. 1985; Kennelly et al. 1984; Lakshmi et al. 1995a, 1995b, 1996b, 1997; Lynn et al. 1984). Because primary amines are easier to oxidize than the acetylated amide products, N-acetylation is considered a detoxification reaction and competes with N-oxidation.

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Also, because amides do not form glucuronides, N-glucuronidation and N-acetylation are also competing pathways. The prevalence of any one of these reactions is species-specific and seems to ultimately determine the potential development of organ-specific cancer. Continued research on the involvement of these main pathways in the metabolism of benzidine and the development of inhibitors of specific reactions could help in the prevention of benzidine-induced toxicity. Other enzymatic pathways in the bioactivation of benzidine, particularly in the bladder, have also been suggested (Flammang et al. 1989; Wise et al. 1984c). Studies to date have also not addressed whether saturation phenomena may come into play in the metabolism of benzidine by one or more of these pathways. Such information would be useful in extrapolating to human exposure situations.

No studies were found regarding excretion in humans or animals after inhalation exposure to benzidine. However, benzidine and acetylated derivatives were detected in the urine of workers exposed to benzidine-based dyes (Dewan et al. 1988; NIOSH 1980c). These dyes are thought to be reduced to free benzidine by azoreductase of the liver and intestinal microflora. Inhalation was presumed to be the major route of exposure, but dermal exposure may also have been a factor. Information regarding excretion in humans after oral exposure to benzidine is quite limited, but urinary free benzidine and various hydroxylated and/or acetylated derivatives have been reported (Englebartz and Babel 1953; Troll et al. 1963). Similar data have also been generated in the rat (Lynn et al. 1984) and monkey (Rinde and Troll 1975). In rats treated with a single oral dose of benzidine, excretion was primarily via the feces, but the percentage excreted in the urine increased with increasing dose (Lynn et al. 1984). The percentage of various benzidine metabolites in the urine was similar in rats treated with the low (0.5 mg/kg) and mid-level (5.0 mg/kg) dose, but changed slightly when the dose was increased to 50 mg/kg. No excretion was detected in expired air. Urinary excretion of benzidine and its metabolites was detected in workers thought to be exposed primarily by the dermal route (Meigs et al. 1951, 1954; Sciarini and Meigs 1961). In rats exposed dermally to radiolabeled benzidine, excretion was again principally via the urine during the first 8 hours, but by 24 hours, was nearly equally divided between feces and urine (Shah and Guthrie 1983). Similar results were found in rats after intravenous exposure (Kellner et al. 1973; Lynn et al. 1984), where it was also shown that virtually all fecal metabolites originate in the liver and are excreted in the bile. After intravenous or intraperitoneal injection of benzidine, excretion data have also been generated for dogs (Kellner et al. 1973; Lakshmi et al. 1990a; Sciarini and Meigs 1958), monkeys (Kellner et al. 1973), and mice (Sciarini and Meigs 1961). Current data support the hypothesis that the exposure route is not a critical variable in benzidine's excretion.

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**Comparative Toxicokinetics.** Current data indicate that benzidine's principal target organ varies from species to species. For example, as noted above, in humans and dogs (and perhaps more weakly in rabbits), the bladder is the principal target organ, whereas in rats, mice, and hamsters, the liver is the primary target. *In vivo* and especially *in vitro* studies have indicated that species vary in cytochrome P-450 content (Neis et al. 1985b), acetylation and deacetylation activity (Bos et al. 1980, 1981; Brouns et al. 1982; Lakshmi et al. 1990a, 1995a, 1995b; Moore et al. 1984; Wang et al. 1990), sulfotransferase activity (Martin et al. 1982), and glucuronidation activity (Babu et al. 1993a, 1994a, 1994b). These findings have helped elucidate the basis for the species-specific carcinogenic response to exposure to benzidine. The principal route of excretion of benzidine is also species dependent, being the feces in rats (Kellner et al. 1973; Lynn et al. 1984) and the urine in dogs (Kellner et al. 1973; Lakshmi et al. 1990a) after intravenous injection. In addition, a dermal study on benzidine-based dyes (Aldrich et al. 1986) reported little absorption of benzidine on the shaved skin of rabbits. Therefore, additional toxicokinetic studies are needed to identify the best animal model for humans. Without further understanding of relevant mechanisms and more detailed data from different species, the rat seems insufficient, and even the dog (a frequently mentioned candidate) has its shortcomings (it cannot acetylate as well as humans and most other species can) in being a model for humans. In addition, more quantitative information, especially on human absorption at occupational exposure levels, would also assist in extrapolating animal data to humans.

**Methods for Reducing Toxic Effects.** No studies were located regarding the mechanism of absorption in humans or animals after inhalation, oral, or dermal exposure to benzidine. However, it might be expected that gastrointestinal absorption of benzidine immediately following oral exposure could be reduced by induced emesis, gastric lavage, saline cathartics, or activated charcoal (Bronstein and Currance 1988; Sittig 1991; Stutz and Janusz 1988). Emesis, however, is contraindicated in obtunded, comatose, or convulsing patients. Also, oils should not be used as a cathartic, as they may enhance the gastrointestinal absorption of benzidine. Saline cathartics should be used with caution in patients with impaired renal function. Following dermal exposure to benzidine, contaminated clothing should be removed and contacted skin washed immediately with soap and water, or the contacted eye immediately irrigated, perhaps with the assistance of proparacaine hydrochloride (Bronstein and Currance 1988; Sittig 1991; Stutz and Janusz 1988). There is no information on the distribution of benzidine following exposure in humans, but studies in animals suggest that benzidine does not tend to accumulate in the body. No studies were located regarding methods for reducing body burden after inhalation, oral, or dermal exposure to benzidine. Human and animal studies indicate that benzidine is excreted fairly rapidly in the urine and/or feces (Lynn et al. 1984; Meigs et al. 1951; Sciarini and Meigs 1961), but much animal

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data suggest that enterohepatic recirculation is involved in prolonging the retention and subsequent metabolism of benzidine (Chipman and Mohn 1989; Lynn et al. 1984). Relatively prompt and repeated administration of activated charcoal (which is sometimes administered to minimize enterohepatic recirculation of persistent chemicals) may therefore be of some benefit in reducing the body burden of benzidine. Benzidine probably produces most of its toxic effects through the binding of oxidized reactive intermediates to nucleic acid and protein target molecules. Currently, there is little that can be done to intervene once binding has occurred. However, several studies have discussed and provided data concerning interrupting the prostaglandin H synthase activation pathway, which is speculated to be of importance especially in bladder and other extra-hepatic tissues (Lakshmi et al. 1990b; Zenser and Davis 1990; Zenser et al. 1980, 1983). It is possible that this enzyme pathway could be inhibited with aspirin or indomethacin, that activation could be inhibited by competitive substrates (propylthiouracil, phenidone, methimazole, MK447), that reactive intermediates (e.g., benzidinediimine) could be reduced back to parent compounds (e.g., benzidine) by reducing agents (vitamin C) or antioxidants (ethoxyquin, butylated hydroxytoluene), or that benzidine and its reactive metabolites could be inactivated and eliminated by conjugation with molecules such as glutathione. Intervention with antioxidant, reducing, and conjugating agents would also be expected to be of some value for inhibiting bioactivation by any of the other potentially involved enzymic pathways. Further investigation of such approaches across animal species and in humans would be useful.

**Children's Susceptibility.** The information on health effects of benzidine in humans is derived mainly from occupational studies. Despite the inherent limitations of these types of studies, there is conclusive evidence that benzidine is a bladder carcinogen in humans (Bi et al. 1992; Bulbulyan et al. 1995; Ferber et al. 1976; Goldwater et al. 1965; Mancuso and El-Attar 1967; Mason et al. 1986; Meigs et al. 1986; Montanaro et al. 1997; Naito et al. 1995; Shinka et al. 1991; Tsuchiya et al. 1975; Vigliani and Barsotti 1962; Xue-Yun et al. 1990; Zavon et al. 1973). Occupational studies have provided also suggestive evidence of carcinogenicity at other tissue sites (Delzell et al. 1989; Morinaga et al. 1982; Okubo et al. 1985; Piolatto et al. 1991; Wu 1988). 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 benzidine, but given the unlikelihood of exposure to benzidine by the general population, such studies do not seem warranted at this time.

There is no information on whether the developmental process is altered in humans exposed to benzidine. No data were located regarding developmental effects of benzidine in animals after any route of exposure. Experiments to determine whether benzidine (or its metabolites) can cross the placenta and/or be

### 3. HEALTH EFFECTS

transferred to offspring via breast milk would be helpful in assessing whether the developmental process might be affected in the offspring of animals exposed to benzidine by a relevant route of exposure. There is some indirect evidence that benzidine or its metabolites can cross the placenta. This evidence is based on the results of a study in which intraperitoneal administration of benzidine to pregnant mice resulted in the induction of micronuclei in the liver of fetuses (Sanderson and Clark 1993). There is no information on whether prenatal or postnatal exposure to benzidine can affect childhood cancer incidence. There are no data to evaluate whether pharmacokinetics of benzidine in children are different from adults. There are no PBPK models for benzidine, but a need for such a model is not apparent at this time. There is no information to evaluate whether metabolism of benzidine in children is different than in adults, but there are some theoretical reasons to suspect that it might be different, including the fact that subsets of metabolic enzymes thought to participate in benzidine metabolism appear to be developmentally regulated (Leeder and Kearns 1997).

Children are highly unlikely to be exposed to benzidine and, consequently, there is no apparent need to develop biomarkers of exposure for children. It is reasonable to assume that biomarkers that could be developed for adults would also be applicable to children. There are no data on the interactions of benzidine with other chemicals in children or adults. There are no pediatric-specific methods to reduce peak absorption for benzidine following exposure, to reduce body burdens, or to interfere with benzidine's mechanism of action, but it is reasonable to assume that exposure avoidance measures should be applied to children as needed.

Child health data needs relating to exposure are discussed in 6.3.1 Identification of Data Needs: Exposures of Children.

#### **3.12.3 Ongoing Studies**

A limited number of ongoing studies concerning health effects and mechanisms of action associated with benzidine have been identified in the Federal Research In Progress database (FEDRIP 2000). These studies, summarized below, may help fill some of the data needs previously identified.

Dr. K.H. Brock, from the Environmental Health Research and Testing, Inc., Durham, North Carolina, is developing cell-mediated activation systems with rodent liver, bladder, kidney, and intestinal cells for benzidine-based dyes in the mouse lymphoma L5178Y TK/assay to determine the metabolite profile of these dyes in the specific tissue. Future studies will determine the capacity of humans cells from these

## 3. HEALTH EFFECTS

tissues to activate benzidine-based dyes to a mutagenic/clastogenic form. This research is sponsored by the Department of Health and Human Services (DHHS).

Dr. T.R. Devereux, from the National Institute of Environmental Health Sciences (NIEHS), National Institute of Health, plans to examine a set of human bladder tumors from a cohort of people occupationally exposed to benzidine by genomic hybridization for gains or losses of genetic material. The research is sponsored by NIEHS.

Dr. G.P. Hemstreet at the University of Oklahoma Health Science Center, Oklahoma City, Oklahoma, plans to continue screening studies in a cohort of workers occupationally exposed to benzidine both to develop a bladder cancer screening approach and to reduce the number of deaths and serious morbidity associated with bladder cancer in this cohort. The research is sponsored by NIOSH.

Dr. G.M. Lower, from BC Research, Gay Mills, Wisconsin, will use sulfamethazine phenotyping to examine well-defined Japanese bladder cancer populations with and without histories of cigarette smoking, and with and without known occupational exposure to benzidine or  $\beta$ -naphthylamine. The results are expected to allow assessment of the relationship between arylamine-induced bladder cancer and the slow acetylator phenotype as a determinant of susceptibility. This research is sponsored by the DHHS.

Dr. T.K. Rao, from Integrated Laboratory Systems, Research Triangle Park, North Carolina, proposes to conduct cell-mediated metabolism/mutagenesis studies to examine cell/tissue/species specificity for a number of bladder carcinogens. Several factors that affect induction and promotion of bladder carcinogenesis will be investigated. This research is sponsored by the DHHS.

Dr. T.V. Zenser at the Veterans' Administration Medical Center, St. Louis, Missouri, is conducting research aimed at elucidating the pathways involved in benzidine metabolism and DNA-adduct formation in humans exposed to benzidine. This research is sponsored by the National Cancer Institute. Under the sponsorship of the Department of Veterans Affairs, Dr. Zenser is also assessing the possible formation of the DNA-adduct, N'-(3'-monophospho-deoxyguanosin-8-yl)-N-acetylbenzidine, by peroxidatic activation of N-acetylbenzidine in workers exposed to benzidine.

## **4. CHEMICAL AND PHYSICAL INFORMATION**

### **4.1 CHEMICAL IDENTITY**

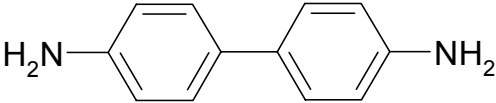
Table 4-1 lists common synonyms, trade names and other pertinent identification information for benzidine.

### **4.2 PHYSICAL AND CHEMICAL PROPERTIES**

Table 4-2 lists important physical and chemical properties of benzidine.

## 4. CHEMICAL AND PHYSICAL INFORMATION

**Table 4-1. Chemical Identity of Benzidine**

Characteristic	Information	Reference
Chemical name	Benzidine	HSDB 1999
Synonym(s)	4,4'-Bianiline; 4,4'-Diphenyldiamine; 4,4'-Diaminobiphenyl; 4,4'-Diphenylenediamine; (1,1'-Biphenyl)-4,4'-diamine; C.I. Azoic Diazo Component 112	IARC 1982a; HSDB 1999
Registered trade name(s)	Fast Corinth Base B	IARC 1982a
Chemical formula	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub>	Lide 1998
Chemical structure		
Identification numbers:		
CAS Registry	92-87-5	Lide 1998
NIOSH RTECS	DC 9625000	NIOSH 1984c
EPA Hazardous Waste	U021	HSDB 1999
OHM/TADS	8100001	HSDB 1999
DOT/UN/NA/IMCO	UN 1885	HSDB 1999
shipping	IMO Class 6.1	HSDB 1999
HSDB	948	HSDB 1999
NCI	C03361	NLM 1988

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



## 4. CHEMICAL AND PHYSICAL INFORMATION

**Table 4-2. Physical and Chemical Properties of Benzidine**

Property	Information	Reference
Molecular weight	184.24	Lide 1998
Color	Grayish-yellow, white or reddish-gray	Lewis 1993
Physical state	Crystalline powder	Budavari et al. 1996
Melting point	120 EC	Lide 1998
Boiling point	401 EC	Lide 1998
Specific gravity (20/4 EC)	1.250	Verschuieren 1983
Odor	No data	
Odor threshold:		
Air	No data	
Water	No data	
pK <sub>a</sub>	4.3 (monoprotonated) 3.3 (diprotonated)	Zierath et al. 1980
Solubility:		
Water at 12 EC	400 mg/L	Verschuieren 1983
at 20 EC	276 mg/L	EPA 1987b
at 25 EC	520 mg/L	EPA 1987b
at 100 EC	9,346 mg/L	Budavari et al. 1996
Organic solvent(s)	200 g/L (boiling alcohol) 20 g/L ether	Budavari et al. 1996 Budavari et al. 1996
Partition coefficients:		
Log K <sub>ow</sub>	1.34	Hansch et al. 1995
Log K <sub>oc</sub>	1.02–4.9	EPA 1981b, 1987b; Johnson and Means 1986
Vapor pressure:		
25 EC	7.0x10 <sup>-7</sup> mmHg	Neely and Blau 1985
20 EC	7.5x10 <sup>-9</sup> mmHg	Schmidt-Bleik et al. 1982
Henry's law constant at 25 EC	5.2x10 <sup>-11</sup> atm·m <sup>3</sup> /mol	Meylan and Howard 1991
Autoignition temperature	No data	
Flashpoint	No data	
Flammability limits	Does not burn or burns with difficulty	HSDB 1999
Conversion factors at 25 EC	1 ppm = 0.133 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 7.52 ppm	IARC 1982a
Explosive limits	No data	

pK<sub>a</sub> = The dissociation constant of the conjugate acid



## 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

### 5.1 PRODUCTION

Benzidine was first prepared in 1845 via the reduction of azobenzene with ammonium sulphide, followed by treatment of the hydrazobenzene with sulfuric acid and treatment with a strong base to release the free benzidine (IARC 1982a). Benzidine can be produced commercially by several methods. The most common is the reduction of nitrobenzene with zinc and sodium hydroxide (Budavari et al. 1996). It can also be produced by the electrolysis of nitrobenzene, followed by distillation, or by the nitration of diphenyl followed by reduction of the product with zinc dust in alkaline solution with subsequent distillation (Lewis 1993). In the production of benzidine-based dyes (dyes characterized as having a biphenyl diazo based linkage that could be derived from benzidine, 3,3'-dichlorobenzidine or a benzidine congener), the benzidine formed as the dihydrochloride salt would not be isolated, but would be reacted directly with so-called chromophore substances to form the dye (EPA 1986c). An estimated production of 500 pounds (227 kg) of benzidine is given for 1983 (EPA 1986c), though this may omit some captive production. In contrast, 10 million pounds (4,720 metric tons) were produced in the United States in 1972 (EPA 1976b).

Benzidine is no longer produced for commercial sale in the United States. In 1973, OSHA regulations effectively banned U.S. production of benzidine. Any benzidine production must be for captive consumption (in-house use), and it must be maintained in closed systems under stringent workplace controls (IARC 1982a). In 1993, the United States International Trade Commission reported that Direct Black 38, a benzidine-based dye, was produced domestically by one manufacturer, but the quantity was not disclosed (USITC 1994).

Although companies that manufacture or process benzidine are required to report maximum quantities stored on-site and quantities released to environmental media or disposed of off-site for the Toxics Release Inventory (TRI), no information has been reported on benzidine from 1987 to 1996. Recent changes have been incorporated into the TRI reporting status of hazardous waste incinerators and waste management companies, and the most recent data indicate that there are four facilities located in California, Illinois, Ohio, and Texas that released minor amounts of benzidine to the environment in 1999. While the absence of TRI data does not confirm that benzidine is not produced or used in the United States since only certain types of facilities are required to report, these TRI results are in keeping with a steadily declining trend in the levels of benzidine produced or used in the United States.

## 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

Commensurate with these results, there are no references on United States-based benzidine production in the latest available edition of SRI's *Directory of Chemical Producers* (SRI 2000).

## 5.2 IMPORT/EXPORT

In 1980, imports of benzidine were estimated at 8,900 pounds (4.08 metric tons) (SRI 1988). Imports of Direct Black 38, the most widely produced benzidine-based dye, were 200,000 pounds (91 metric tons) in 1978, up sharply from 1976 and 1977; total benzidine-based dye imports in 1979 were 469,000 pounds (213 metric tons), up sharply over the decade (USITC 1986). In recent years, there have been no reported imports of benzidine itself due to concerns over its toxicity. Benzidine-based dyes may still be imported into the United States, although no quantitative data on the amount imported are available (Anonymous 1999; Pfaltz and Bauer 1999; Weber 1991). The benzidine-based dyes, Direct Black 38 and Direct Red 28, are imported into the United States and can be purchased commercially (Aldrich 1998; Anonymous 1999; Pfaltz and Bauer 1999).

## 5.3 USE

The predominant use of benzidine was for the manufacture of dyes, especially azo dyes in the leather, textile, and paper industries. The Color Index lists over 300 colors of benzidine-based dyes, some 18 of which were found to be commercially available in the United States (AATCC 1984; Gregory 1984). Eleven of these 18 dyes were domestically manufactured. It is unclear whether these dyes were manufactured from benzidine or benzidine congeners such as o-tolidine or o-dianisidine. Some of these dyes, especially direct browns, greens, and blacks, were available as consumer products in the 1970s (Jenkins 1978). Orzeck and Gregory (1981) listed 65 home dyes that contained benzidine or benzidine congeners. Access to such dyestuffs for home use is no longer available, just as remaining industrial uses must embody a stringently controlled captive consumption approach (IARC 1982a). Benzidine or related benzidine congeners may be found as minor impurities in a variety of azo dyes used in coloring prepared for food items.

Benzidine was once used in clinical laboratories for the detection of blood, but this application has been discontinued due to safety concerns and the availability of more specific methods. Other uses listed for benzidine include as a rubber compounding agent, for the manufacture of plastic films, to detect H<sub>2</sub>O<sub>2</sub> in milk, for security printing, and for the determination of nicotine. Benzidine was also used in small quantities for the manufacture of organic chemicals and as a laboratory agent (Lawler 1977). While

## 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

diaminobenzidine and related dyes derived from aromatic amines are still in common use as stains in microscopy and similar lab applications (Lunn and Sansone 1991), information is not available to quantify the levels of benzidine (likely as trace impurities) contained in such lab preparations.

**5.4 DISPOSAL**

Formerly, benzidine may have been released during production or processing, and also from its presence in dyes. Currently, regulations restricting the production and use of benzidine limit the quantity of material discharged. The EPA has listed benzidine as a "priority" chemical that can cause cancer in humans and is subject to rigid inspection and control. The amount of benzidine that can be allowed in waste transported to waste disposal sites is 0.1 ppm (EPA 1980b). Any material products, residues, or container liners containing or in contact with benzidine are considered toxic wastes according to the Resource Conservation and Recovery Act (RCRA). Rotary kiln or fluidized-bed incineration may be appropriate disposal methods for benzidine (HSDB 1999). As a minor impurity in other waste materials, benzidine may still be released in effluent waste water discharges by the dye industry. According to the TRI, benzidine was not transferred to landfills from 1987 to 1999 (TRI87 1989; TRI90 1992; TRI92 1994; TRI99 2001). However, the most recent TRI data indicates that waste containing benzidine has been burned at hazardous waste incinerators (TRI99 2001). It should be noted, however, that many types of commercial facilities are exempt from TRI reporting requirements.



## 6. POTENTIAL FOR HUMAN EXPOSURE

### 6.1 OVERVIEW

Since benzidine may only be produced for captive use in the United States, its direct release to the environment is expected to be low. Benzidine-based dyes may still be imported into the country (Anonymous 1999; Pfaltz and Bauer 1999; Weber 1991). These dyes can undergo microbial degradation and release free benzidine to the environment (Weber 1991; Yoshida et al. 1981). In the past, benzidine's production and use may have led to its release to the environment from waste waters and sludges. Relatively high benzidine levels have been detected in soils and water in the vicinity of industrial sources (Boyd et al 1984; Zhao et al. 1998).

Benzidine is a moderately persistent compound in the environment, but it may readily partition from solution to soils and sediments, which reduces the potential for human exposure. In the atmosphere, benzidine exists in both the particulate and vapor phases (Kelly et al. 1994). Particulate-phase benzidine may be removed from the atmosphere by wet and dry deposition, while vapor-phase benzidine will be readily degraded by reaction with photochemically-produced hydroxyl radicals. Benzidine may undergo direct photolysis in the atmosphere, but the kinetics of this reaction are unknown. In water, benzidine does not significantly volatilize or hydrolyze, but it may be oxidized by cations. Benzidine may undergo photolysis in sunlit surface waters. Benzidine may be strongly adsorbed from solution by soils, sediments, and clays, depending on the pH of the soil-water system. It may also be oxidized at clay surfaces. Benzidine does not seem to be readily biodegradable in soil, but does degrade at low concentrations in acclimated sludges. Benzidine may be bioconcentrated by aquatic organisms, but it appears that the extent of concentration is not significant and that it will not be transferred through the food chain to higher levels.

The direct risk of benzidine exposure to the general population appears to be low. Concern for human health is primarily focused on populations living near former manufacturing or disposal sites where benzidine or benzidine-based dyes may have been manufactured or disposed of. Impurities contained in certain food dyes may also be metabolized once inside the body and release free benzidine.

Benzidine has been identified in at least 28 of the 1,585 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2001). However, the number of sites

## 6. POTENTIAL FOR HUMAN EXPOSURE

evaluated for benzidine is not known. The frequency of these sites within the United States can be seen in Figure 6-1.

## 6.2 RELEASES TO THE ENVIRONMENT

Benzidine is a synthetic chemical and has not been reported to occur naturally in the environment (EPA 1986c). It is released to the environment only through industrial production and use, and through the degradation of benzidine-based dyes. Since 1976, releases of benzidine have decreased because it is only produced for captive consumption in the United States (see Section 5.1).

### 6.2.1 Air

No data were located documenting current benzidine emissions to the atmosphere. Prior to 1974, benzidine and its derivatives were manufactured and used in open systems that permitted atmospheric releases at the workplace. Based on the current regulations restricting the production and use of benzidine (see Table 8-1), atmospheric emissions of benzidine are expected to be low.

No emissions of benzidine to air from manufacturing and processing facilities in the United States from 1987 to 1996 were reported on the TRI (TRI87 1989; TRI90 1992; TRI92 1994; TRI96 1999). It was reported that 13 pounds of benzidine were released to the air from four facilities in the United States in 1999 (TRI99 2001). These emissions resulted from the incineration of hazardous waste and not from the manufacture, production, or use of benzidine.

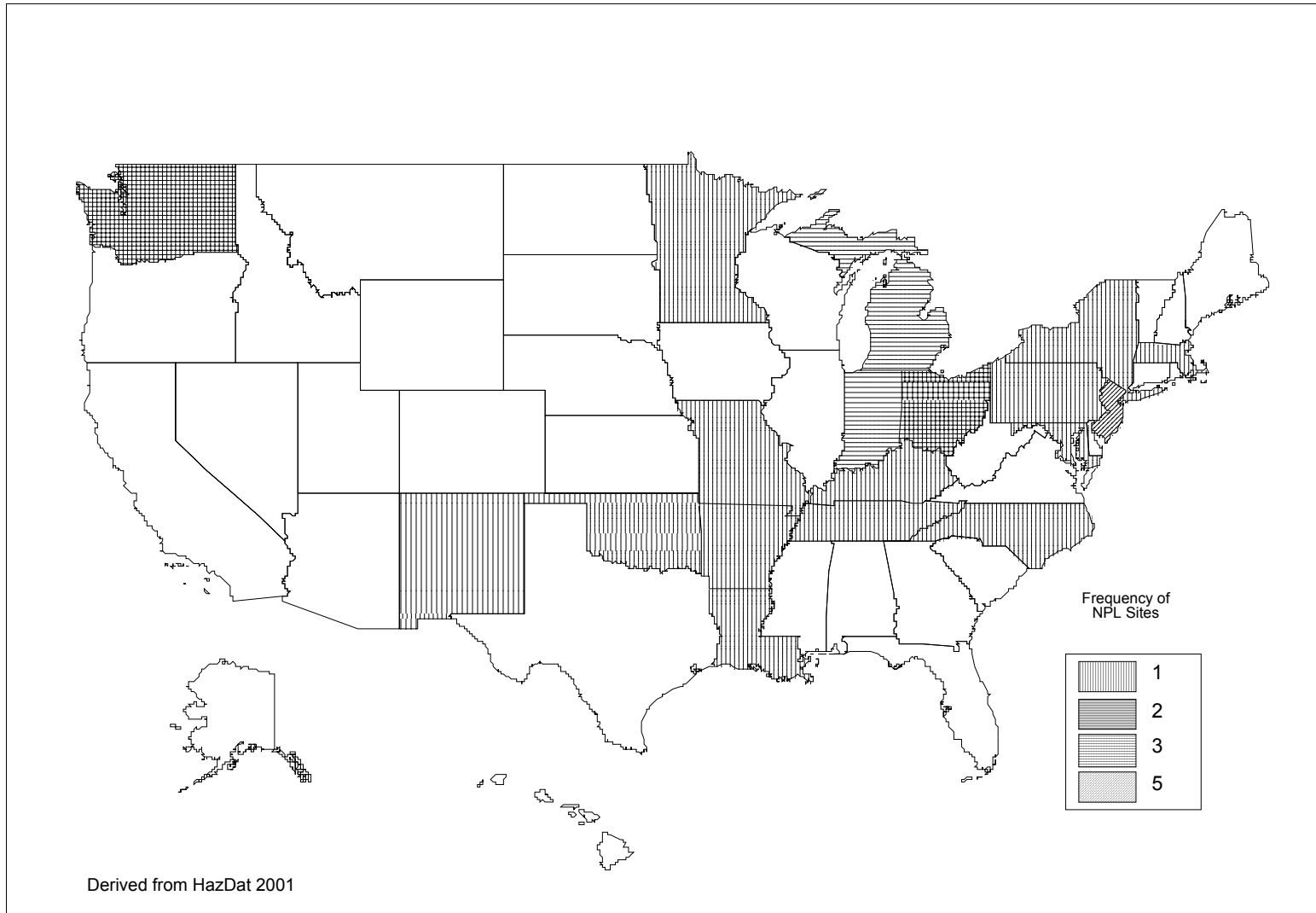
Benzidine was not identified in air samples collected from the 28 NPL hazardous waste sites where it was detected in some environmental media (HazDat 2001).

### 6.2.2 Water

No data were located documenting current benzidine emissions to water. In the past, benzidine was released into the environment in waste waters generated by its production or its use in the synthesis of azo dyes (EPA 1980a). Industrial reports have claimed that benzidine discharges at any production facility generally did not exceed approximately 0.45 kg/day (EPA 1979d), and that the discharged benzidine concentrations in the dye wastes were generally less than 10 ppm. Benzidine was estimated to be a



Figure 6-1. Frequency of NPL Sites with Benzidine Contamination



## 6. POTENTIAL FOR HUMAN EXPOSURE

potential hazard only in the vicinity of dye and pigment plants (EPA 1980a). Waste effluent from factories where textiles were dyed with benzidine-based dyes contained an average benzidine concentration of 3.5 ppb ( $\mu\text{g/L}$ ), effluents from a leather factory contained an average of 0.25 ppb ( $\mu\text{g/L}$ ) benzidine (EPA 1979b), and effluents from a foundry contained up to 10 ppb ( $\mu\text{g/L}$ ) benzidine (EPA 1986c). Benzidine was detected in 1.1% of 1,235 effluent samples reported on the Storage and Retrieval (STORET) database maintained by EPA (Staples et al. 1985). The median concentration of all samples was less than 10 ppb ( $\mu\text{g/L}$ ). These STORET results must be used with some caution, since many of the analysis methods through the early 1980s used techniques that could not distinguish between benzidine and a variety of related aromatic amines and metabolites (EPA 1978c). In some cases, therefore, the STORET data may overestimate the reported benzidine levels. Benzidine was detected in the waste water of a dye manufacturing plant in Dalian, China, at a concentration of 23 ppb ( $\mu\text{g/L}$ ) (Zhao et al. 1998).

It was demonstrated that anaerobic sediment-water systems were able to biodegrade benzidine-based dyes to benzidine (Weber 1991). Direct Red 28 was degraded in an anaerobic sediment-water slurry and about 5% of the degradation products were shown to be benzidine (Weber 1991). Although the amount of benzidine produced was not quantified, the dye Deep Black EX was shown to biodegrade in river water, and benzidine was a metabolite (Yoshida et al. 1981).

Benzidine was not reported to be discharged to surface water or by underground injection from manufacturing and processing facilities in the United States from 1987 to 1999 according to TRI records (TRI87 1989; TRI90 1992; TRI92 1994; TRI96 1999; TRI99 2001). Since only certain types of facilities are required to report, it is possible that some benzidine has been released to water in small quantities or from other types of facilities.

Benzidine was identified in surface water collected at 5 sites and in groundwater collected at 10 of the 28 NPL hazardous waste sites where it was detected in some environmental media (HazDat 2001).

### 6.2.3 Soil

No data were located documenting current benzidine emissions to soil surfaces. There is a potential for soils to become contaminated with benzidine if waste water treatment sludge or industrial wastes containing benzidine or benzidine-based dyes are land disposed. The dye Deep Black EX was shown to biodegrade in soils, and one of the degradation products was benzidine (Yoshida et al. 1981). The improper disposal of industrial sludges from a benzidine-manufacturing plant in Michigan resulted in soil,

## 6. POTENTIAL FOR HUMAN EXPOSURE

groundwater, and surface water contamination (Boyd et al. 1984). According to TRI, benzidine was not discharged to land from manufacturing and processing facilities in the United States from 1987 to 1999 (TRI87 1989; TRI90 1992; TRI92 1994; TRI96 1999; TRI99 2001).

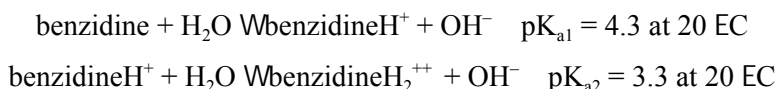
Benzidine has been identified in soil samples collected from 9 sites and in sediment samples from 4 of the 28 NPL hazardous waste sites where it was detected in some environmental media (HazDat 2001).

### 6.3 ENVIRONMENTAL FATE

#### 6.3.1 Transport and Partitioning

Benzidine is expected to exist in both the particulate and vapor phases in the ambient atmosphere (Kelly et al. 1994). Particulate-phase benzidine is subject to atmospheric dispersion, gravitational settling, and wash-out by rain. Vapor-phase benzidine reacts rapidly with photochemically generated hydroxyl radicals, and thus, its transport in the atmosphere is limited. The Henry's law constant for benzidine is estimated as  $5.2 \times 10^{-11}$  atm-m<sup>3</sup>/mol using a fragment constant estimation method (Meylan and Howard 1991). Based on this Henry's law constant, volatilization from moist soils and water surfaces is not expected to be an important environmental fate process and only minor amounts of benzidine can be expected to partition to the atmosphere.

In water, benzidine may be adsorbed by 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. EPA (1981b) calculated a  $K_{oc}$  value for benzidine of 10.5, based on an octanol-water partition coefficient ( $K_{ow}$ ) of 21.9. This relatively low value implies that benzidine is a "very highly mobile" compound in saturated soil-water systems (Roy and Griffin 1985). However, benzidine is a weak base in solution, and exists in both neutral and cationic forms. Written as hydrolysis reactions, the amine groups on the benzidine molecule may be protonated (Korenman and Nikolaev 1974), as follows:



## 6. POTENTIAL FOR HUMAN EXPOSURE

At low pH, as in the case of acidic soils, benzidine will partially exist in the protonated form and can bind strongly to cation exchange sites in soils. As the pH increases, the proportion of cationic benzidine decreases, and the extent of adsorption via Coulombic interactions would decrease. This was demonstrated by Zierath et al. (1980), who found that the adsorption of benzidine by soils and sediments was controlled primarily by the concentration of the ionized species, which in turn, was highly correlated with pH. As the pH decreased, the magnitude of adsorption increased because of the increase in cationic forms which, via Coulombic interactions, would have a greater affinity for charged surfaces than would the neutral form. A recent study has supported these conclusions. Sorption of benzidine to three silt clay loams was measured from  $\text{CaCl}_2$  electrolyte solutions at pH ranges of 4.4–7.2 (Lee et al. 1997). It was determined that the magnitude of sorption increased with decreasing pH, decreased with increasing  $\text{CaCl}_2$  electrolyte concentration, and was affected by competition with other organic amines that competed with benzidine for cation exchange sites (Lee et al. 1997).

Benzidine is an aromatic amine that can also form covalent bonds with humic material in soils (Lee et al. 1997; Ononye and Gravel 1994; Ononye et al. 1989). This reaction usually involves imine formation between the amine group of benzidine and a carbonyl group of soil humic material. This reaction is reversible, and is partly responsible for the rapid binding of primary aromatic amines in soils. Benzidine may also undergo another type of binding involving a nucleophilic substitution reaction with quinone type residues in the soil matrix. This type of adsorption may be irreversible or only slowly reversible. Lee et al. (1997) have provided evidence that benzidine undergoes both of these reactions in soil, but the primary mechanism for the initial adsorption of benzidine is through the interaction of the protonated species with cation exchange sites in the soil matrix. The reported  $K_{oc}$  for benzidine on estuarine colloidal organic matter was 3,430 at pH 7.9 (Means and Wijayarathne 1989), indicating that benzidine will adsorb strongly to suspended solids and sediment in the water column.

Four aquatic organisms were exposed to radiolabeled benzidine in laboratory model ecosystems for 33 days by Lu et al. (1977). Bioconcentration factors (BCFs) were calculated for each organism: fish, 110; mosquito, 1,180; snail, 370; and algae, 160. In a 42-day experiment, bluegills were exposed to  $^{14}\text{C}$ -benzidine in a flow-through tank. The BCF was 44 for the edible portion of the fish (EPA 1978c). Although benzidine does bioconcentrate to some degree, it is not strongly bioaccumulated by the lower forms of aquatic organisms or transferred through food chains to higher trophic levels (EPA 1986c).

## 6. POTENTIAL FOR HUMAN EXPOSURE

**6.3.2 Transformation and Degradation****6.3.2.1 Air**

Benzidine is expected to exist in both the particulate and vapor phases in the ambient atmosphere (Kelly et al. 1994). The rate constant for the vapor-phase reaction of benzidine with photochemically-produced hydroxyl radicals has been estimated as  $1.54 \times 10^{-10}$  cm<sup>3</sup>/molecule-sec at 25 EC using a structure estimation method (Meylan and Howard 1993). This corresponds to an atmospheric half-life of about 2.5 hours at an atmospheric concentration of  $5 \times 10^5$  hydroxyl radicals/cm<sup>3</sup> (Meylan and Howard 1993). Particulate-phase benzidine is not expected to react with photochemically-produced hydroxyl radicals, and therefore, will not be readily degraded in the atmosphere. Benzidine absorbs light greater than 290 nm, and thus, may also undergo direct photolysis in the environment (Banerjee et al. 1978), but the kinetics of this reaction are unknown.

**6.3.2.2 Water**

Studies conducted by the Synthetic Organic Chemical Manufacturer's Association (SOCMA) indicated that the half-life of benzidine in dilute aqueous solution was 4 hours (EPA 1976a). However, neither reaction mechanisms nor degradation products were determined, and only the disappearance of benzidine itself from solution was an empirical observation.

Other than the hydrolysis (or protonation) of the amine groups, no data were located to suggest that the hydrolysis of benzidine is significant (EPA 1979c). A hydrolysis-rate constant of 0/mol-hour has been proposed (EPA 1981b, 1987c).

There is some evidence that benzidine may degrade in water by oxidation, but actual data on reaction rates were not available. Based solely on reaction rates with peroxy radicals, the half-life of benzidine in water has been estimated to be 100 days (range of 10–1,000 days) (EPA 1978c). Based on oxidation rates of similar compounds, the oxidation rate of benzidine with peroxy radicals was estimated to be approximately  $1.1 \times 10^8$ /mol-hour (EPA 1981b). However, there is a paucity of data to actually demonstrate that these reactions are environmentally significant. The available literature, therefore, offers widely disparate findings (e.g., EPA 1976a; EPA 1978c) on the fate of benzidine in water. Since the SOCMA studies were based in large measure on lake water samples, one possible explanation might be the tendency of benzidine to oxidize in the presence of certain clay minerals as described below in

## 6. POTENTIAL FOR HUMAN EXPOSURE

Section 6.3.2.3 or to adsorb to suspended solids as described in Section 6.3.1. Other SOCMA test data involved chlorinated water or vigorously aerated water samples, which could have led to rapid oxidation of the benzidine parent materials (EPA 1976a). It was determined that the photolysis half-life of benzidine in methanol was about 2 hours when irradiated at 254 nm (Lu et al. 1977). Furthermore, it was determined that the disappearance quantum yield for benzidine at 300 nm was essentially identical to the disappearance quantum yield at 254 nm (Banerjee et al. 1978). Based upon this data, it is reasonable to conclude that photolysis of benzidine in sunlit surface waters may occur.

**6.3.2.3 Sediment and Soil**

Benzidine may be oxidized when mixed with some clay minerals in solution (Lahav and Raziell 1971; Solomon et al. 1968; Tennakoon et al. 1974a, 1974b; Theng 1971). When aqueous solutions containing benzidine are mixed with montmorillonite, pyrophyllite, hectorite, muscovite, illite, and kaolinite, the solution may turn blue. Benzidine blue, a monovalent radical cation, may form by the oxidation of benzidine by iron(III) in the silicate lattice and by aluminum(III) at crystal edges.

Previous assessments (e.g., EPA 1979c) generalized that cations in solution oxidize benzidine. However, it should be noted that these oxidation observations were made with benzidine-clay slurries. It has not been shown that ambient cations in natural or waste water will significantly oxidize benzidine. The concentrations of iron(III) and aluminum(III) in natural systems are often controlled by sparingly soluble hydroxide and oxide solid phases.

It has not been demonstrated that benzidine biodegrades in natural waters, and it may not readily biodegrade in soil. In one study, Lu et al. (1977) mixed a sample of Drummer soil with 100 µg of benzidine, and incubated it at 26.7 EC. After 4 weeks, only 20.6% of the compound remained in the soil. However, Graveel et al. (1985) generalized that the half-life of benzidine in soil may range from 3 to 8 years. Based on <sup>14</sup>CO<sub>2</sub> evolution, benzidine decomposition averaged only 2% after 84 days of incubation with three soils (a Typic Hapudalf and two Typic Argiaqualls). Additional experiments indicated that after 365 days of incubation, approximately 8–12% of the added benzidine (53.4 µmol/kg dry soil) evolved as <sup>14</sup>CO<sub>2</sub> (Graveel et al. 1986). It should be noted that biodegradation when measured by gas evolution may be a conservative estimate of the extent of decomposition. This technique does not account for carbon that is incorporated into the biomass or the soil organic matter, or if the compound is only partially metabolized (Graveel et al. 1986). Since benzidine may be found in waste water treatment sludges, studies have been conducted to assess whether benzidine would be biodegraded in conventional

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treatment systems. Benzidine was found to be biodegraded by two activated waste water sludges (Baird et al. 1977). The biodegradation of benzidine tended to be greater at lower benzidine concentrations, ranging from 35–88% depletion at 10 mg/L, 11–93% depletion at 20 mg/L, 21–60% depletion at 40 mg/L, and 9–55% depletion at 100 mg/L after a contact time of 6 hours. Little is known about the toxicity of benzidine to microorganisms; higher concentrations may inhibit bio-oxidation (EPA 1978c).

The extent of biodegradation depended on the concentration of the benzidine in aerobic growth reactors using waste water sludges (Tabak and Barth 1978). Using lower benzidine concentrations and longer contact times, the authors found that benzidine degraded to a greater extent. At concentrations of 0.5 and 1 mg/L, biodegradation was nearly complete after a contact time of 1 week, and was complete after a contact time of 3 weeks. At concentrations of 5 and 10 mg/L, 94 and 77%, respectively, of the benzidine was biodegraded after a contact time of 7 weeks. Acclimated sludges can completely degrade continuous low doses (#1 mg/L) of benzidine. They further concluded that ambient concentrations that are <1 than 1 mg/L will be degraded in natural ecosystems. Possible biodegradation products included 4,4'-dihydroxybiphenyl, N-hydroxybenzidine, 4-hydroxy-phenylamine, 4-aminobiphenyl, 3-hydroxybenzidine, 4-amino-4'-nitrobiphenyl, aniline, N,N'-dihydroxybenzidine, phenol, 3-3'-dihydroxybenzidine, and catechol (Baird et al. 1977; Tabak and Barth 1978). The environmental fate and pathways of degradation by products have not been assessed.

Laboratory studies of sediment-water systems suggest that the breakdown of various benzidine-based dyes can liberate benzidine (Weber 1991). Up to 5% of the initial amount of benzidine-dye materials may be transformed to benzidine, especially in anaerobic sediments. At lower pHs, however, sorption to sediment particulates was enhanced, and the degree of transformation diminished substantially. Yoshida et al. (1981) studied the disappearance of Deep Black EX from soil surfaces, and noted that benzidine was one of the products of microbial degradation. It was further noted that the resultant benzidine did not biodegrade in the soil after 9 weeks. However, no quantitative data on the conversion of the dye to benzidine was reported.

#### 6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Benzidine does not occur naturally (IARC 1982a), and has been detected only infrequently and at low concentrations in environmental media. The production and use of benzidine-based dyes have decreased in the last 30 years while environmental regulations have been implemented to reduce the release of benzidine to the environment. Concentrations of benzidine still remaining in the environment may be at

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or below current detection limits or adsorbed to sediments, making it difficult to quantify the amount of benzidine in various environmental media.

**6.4.1 Air**

Monitoring data for benzidine concentrations in ambient air were not located (EPA 1978c; EPA 1979d, 1986c; Kelly et al. 1994).

**6.4.2 Water**

Benzidine is rarely detected in ambient water. It has been analyzed for, but not detected, in several systems (Howard 1989). The median concentration of benzidine in ambient water was less than 10 µg/L, and was detectable in only 1 of 879 samples reported in the STORET database (Staples et al. 1985). In 1990, benzidine was detected at maximum concentrations of 240 µg/L (on-site) and 19 µg/L (off-site) in groundwater at the Nyanza hazardous waste site in Ashland, Massachusetts (ATSDR 1994). This site was formerly the location for Nyanza Company, one of the first and largest dye manufacturers in the United States.

**6.4.3 Sediment and Soil**

Benzidine was not detected in any of the 3,240 sediment sample analyses reported on the STORET database (Staples et al. 1985). Benzidine was not detected in 21 samples from 7 sites in the Buffalo River upstream and downstream from the Allied Chemical plant where benzidine was believed to have been discharged (EPA 1976a).

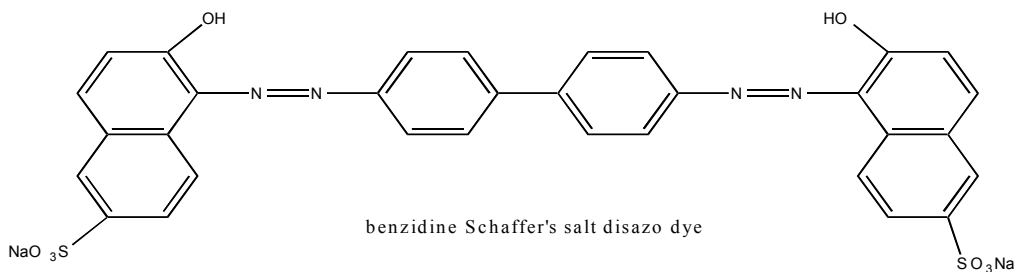
**6.4.4 Other Environmental Media**

No data were located documenting the occurrence of benzidine in foods. An analysis of data from the STORET database indicated that benzidine was not detected in any of the 110 biota samples reported (Staples et al. 1985). Exposure to benzidine through most food products is highly unlikely (EPA 1986c). It is possible that low levels of benzidine or other impurities that can be metabolized to benzidine once inside the body may be found in synthetic coloring agents added to some foods. Although the Delaney Clause of the Food, Drug, and Cosmetic Act bans the use of all carcinogenic agents in food, various synthetic dyes (including those containing small amounts of benzidine-based dye materials and some



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trace amounts of benzidine impurities) are still in use (NIOSH 1980c). The benzidine concentration in food colorants is limited to 1 ppb by the FDA (see Table 8-1). It was recently determined that an impurity thought to be benzidine Schaeffer's salt disazo dye (shown below), was contained in 34 of 67 lots of FD&C Yellow No. 6 (Sunset Yellow FCF; Colour Index No. 15985) at concentrations of 11–941 ppb (Peiperl et al. 1995).



It is believed that this compound may be reduced in the intestine to release free benzidine (Peiperl et al. 1995). This compound arises during the manufacture of dyes due to free benzidine present as an impurity in the sulfanilic acid starting material. More recent data have also supported these findings. Free and bound benzidine was detected in 7 out of 8 samples of FD&C Yellow No. 6 (Sunset Yellow FCF) manufactured from 1991-1998 at concentrations ranging from 45 to 246 ng/g (Lancaster and Lawrence 1999). Free and bound benzidine was also detected in 2 of 9 samples of tartrazine manufactured from 1990-1998 at concentrations of 8 and 20 ng/g (Lancaster and Lawrence 1999).

Of 26 samples of benzidine-based dyes produced in the United States in the late 1970s, 17 samples contained benzidine concentrations ranging from 1 to 4 mg/kg, 8 samples contained from 10 to 20 mg/kg, and 1 sample was measured at 270 mg/kg (NIOSH 1980c). Although no data are available on the rates of azo reduction of benzidine-based dyes in environmental media, the dye called Direct Black 38 is known to be readily reduced in solution (Gregory 1984; Orzeck and Gregory 1981).

## 6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Based on the available data, the potential for benzidine exposure via environmental media (air, soil, water, or food) is low. Certain compounds found as impurities in azo food dyes have the potential to be converted to benzidine once inside the body, and represent a possible source of human exposure. There may remain a risk of benzidine exposure to populations near hazardous waste sites from the former disposal of benzidine or benzidine-based dyes.

## 6. POTENTIAL FOR HUMAN EXPOSURE

Since benzidine is no longer commercially produced or used in the United States, the potential for occupational exposure to this compound is low. Workers that are involved in the in-house production of benzidine may be occupationally exposed via dermal contact and inhalation of benzidine vapors or dust particles. In the past, air concentrations at different locations in a benzidine manufacturing plant ranged from 0.007 to 17.6 mg/m<sup>3</sup> (Howard 1989). Benzidine was also reported in the urine of exposed workers at concentrations ranging from 1 to 112 µg/L (Howard 1989). Benzidine has also been detected in the urine of workers in India exposed to Direct Black 38 at concentrations of 2.4 to 362.5 µg/L (Dewan et al. 1988). Workers that are employed at hazardous waste sites may also be exposed to benzidine if it is present. No data were located on current levels of occupational exposure in the United States since, as noted earlier, recent information indicates that benzidine and benzidine-based dyes are no longer used or marketed in significant quantities by dye manufacturers in the United States.

**6.6 EXPOSURES OF CHILDREN**

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in 3.6 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, 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, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

At one time, benzidine-based dyes were available for home dyeing of fabrics, and accidental ingestions were reported to have occurred in children (Orzeck and Gregory 1981). These home dyes have since been taken off the market and the potential exposure risks are virtually non-existent today. There is no other evidence to indicate that children are directly exposed to benzidine. There are no measurements of benzidine or its metabolites in amniotic fluid, meconium, cord blood or neonatal exposure that indicate prenatal exposure. Furthermore, there are no known studies of benzidine or its metabolites in breast milk.

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Although not documented, children residing near hazardous waste sites where benzidine or benzidine-based dyes were disposed of may be exposed to benzidine via pica (dirt eating) if benzidine is present in the soil. Playing in contaminated soil could also lead to dermal exposure. Even though benzidine volatilizes and biodegrades slowly from soil surfaces, the likelihood of exposure from pica is considered low since benzidine has only been detected in 28 of the 1,585 hazardous waste sites that have been proposed for inclusion on the EPA (NPL) (HazDat 2001). Impurities in certain food dyes such as FD&C Yellow No. 6 can be metabolized to benzidine inside the body (Lancaster and Lawrence 1999; Peiperl et al. 1995). Since this dye is used to color medications, soft drinks, and candies, children may be indirectly exposed to benzidine through the ingestion of compounds that contain this dye. It is unknown whether there is a difference between children and adults in their weight adjusted intake of benzidine.

Since benzidine is no longer produced or used commercially in the United States, exposure to benzidine from parents' work clothes, skin, hair, tools, or other work-related items is unlikely.

### 6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Populations living or working near hazardous waste sites at which benzidine was detected may be exposed to higher levels than the general population. Persons residing near or employed at hazardous waste incinerators may be subject to small amounts of benzidine released to air. Workers in facilities (if any) that manufacture benzidine or benzidine-based dyes for in-house use may also be exposed. During the use of benzidine-based dyes, the greatest potential for exposure would be expected to be among workers who routinely handled the dry powders, which may be readily transformed to an aerosol and inhaled (NIOSH 1980a). Other occupations with potential exposure risks are medical or laboratory technicians where stains are used that may contain benzidine as a trace impurity. During the period 1981–1983 when these types of workplace exposures were still possible, it was estimated that up to 15,554 workers might potentially have been exposed (NIOSH 1990). Of these workers, 830 were in industrial finishing plants, and the remainder were in industries associated with laboratory or medical work. These most recent NIOSH numbers are not based on actual measurements, but rather were extrapolations of surveys, and the precise numbers are best used to make rough estimates of occupations and activities where risks may exist. In light of regulatory changes that went into effect during the 1980s, these NIOSH figures clearly do not provide an accurate basis from which to project current workplace exposure risks.

## 6. POTENTIAL FOR HUMAN EXPOSURE

Workers in the United States currently wear protective equipment to eliminate skin contact with or inhalation of benzidine-containing materials. Worker exposures depend on the specific operations of the facility, but no information on current occupational exposure levels was located.

## 6.8 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 benzidine 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 benzidine.

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.

### 6.8.1 Identification of Data Needs

**Physical and Chemical Properties.** The physical and chemical properties necessary to evaluate the environmental fate of benzidine have been measured or estimated (EPA 1987b; Meylan and Howard 1991; Neely and Blau 1985; Schmidt-Blelk et al. 1982; Zierath et al. 1980). However, estimated properties may have a degree of uncertainty; a measured value for the Henry's law constant, for example, would allow more accurate prediction of the environmental fate of benzidine.

**Production, Import/Export, Use, Release, and Disposal.** Since 1973, benzidine has only been produced in the United States for captive consumption (EPA 1986c). No data were located on current domestic producers, production volumes, or import/export volumes of benzidine-based dyes, but recent information suggests that neither benzidine nor the dyes are currently produced in significant quantities in the United States, although importation of benzidine-based dyes may be continuing (Anonymous 1999; Pfaltz and Bauer 1999; Weber 1991). Ascertaining the quantity of these dyes that are imported would be

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useful in determining the potential for human exposure to benzidine. The most recent TRI has reported small releases of benzidine emitted to the air as a result of incineration of hazardous waste (TRI99 2001).

**Environmental Fate.** Benzidine generally partitions into the soil or sediment in the environment and its mobility decreases with decreasing pH (Lee et al. 1997; Ononye and Gravel 1994; Ononye et al. 1989). It has been demonstrated that clays can oxidize benzidine (Lahav and Raziell 1971; Solomon et al. 1968; Tennakoon et al. 1974a, 1974b; Theng 1971), but more information regarding the biodegradation of benzidine in water, soil, and sediment is necessary. It has been shown that benzidine may degrade in sewage treatment systems (Baird et al. 1977), but research on natural ecosystems would be useful. Also, the nature and environmental fate of degradation by products warrants future study. Information on the environmental degradation of benzidine-based dyes to benzidine is also essential.

**Bioavailability from Environmental Media.** It is generally agreed that benzidine base and salts can be readily absorbed through the intact human skin and through inhalation (Ferber et al. 1976; Meigs et al. 1951, 1954; Zavon et al. 1973), but no quantitative studies were located regarding absorption in humans or animals after oral exposure to benzidine. Studies on the bioavailability of benzidine are needed to determine its risks of entering ecological food chains and potential human exposures. No studies were located regarding the bioavailability of benzidine from environmental media. The lack of data does not necessarily indicate a lack of bioavailability.

**Food Chain Bioaccumulation.** Limited data have shown that benzidine can bioconcentrate in aquatic organisms (Lu et al. 1977). Studies are needed on the potential for benzidine to bioaccumulate in food chains since this would increase the risks of human exposures. No studies were located regarding the food chain accumulation of benzidine from environmental media. It would be useful to know if benzidine does bioaccumulate in the food chain, and whether there is a potential impact on human exposure levels.

**Exposure Levels in Environmental Media.** Benzidine has infrequently been detected in the environment (ATSDR 1994; Staples et al. 1985); however, no estimates have been made of the potential human intake of benzidine from environmental media. There is a need to further determine the ambient concentrations of benzidine and/or benzidine-based dyes in the atmosphere, surface water, groundwater, and soil. However, since benzidine is an anthropogenic substance of limited production, the expected concentrations would be at or below analytical detection limits, making it difficult to estimate general benzidine levels in the environment.

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Further studies regarding impurities in azo food dyes that have the potential to be metabolized to benzidine once inside the body are needed. Identifying these impurities and monitoring the concentrations of these compounds in food dyes are necessary in order to determine the potential impact on humans.

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

**Exposure Levels in Humans.** No measurements of benzidine in human tissues have been located for the general population or for those residing near hazardous waste sites. This information is necessary for assessing the need to conduct health studies on these populations. Benzidine has been detected in the urine of workers who were involved in the production of dyes (Dewan et al. 1988). There is a need for further studies on benzidine and benzidine based dyes to document the degree to which exposures to human populations may still be taking place. Impurities found in certain dyes used to color foods may be broken down to benzidine once inside the body. Accurate data on the level of these impurities in food dyes and a better understanding of the amount of dyed foods ingested by the general population will provide a better understanding of the amount of benzidine that humans are exposed to.

**Exposure Levels in Children.** There is no information available on the exposure of children to benzidine and since it is no longer manufactured or produced in the United States, it is unlikely that children will be exposed to this compound. There do not appear to be unique exposure pathways for children other than pica, but since benzidine is rarely found in the environment, the potential for exposure is considered low. It is not known whether children are different in their weight-adjusted intake of benzidine and no childhood specific means to decrease exposure have been identified. Impurities found in certain dyes used to color foods may be broken down to benzidine once inside the body. Accurate data on the level of these impurities in food dyes and a better understanding of the amount of dyed foods ingested by children will provide a better understanding of the amount of benzidine children are exposed to.

Child health data needs relating to susceptibility are discussed in Section 3.12.2 Identification of Data Needs: Children's Susceptibility.

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**Exposure Registries.** No exposure registries for benzidine 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.

**6.8.2 Ongoing Studies**

No information could be identified on ongoing studies related directly to the fate and transport of benzidine.





## 7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring benzidine, its metabolites, and other biomarkers of exposure and effect to benzidine. 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.

### 7.1 BIOLOGICAL MATERIALS

Several investigators have described means for the determination of benzidine and its metabolites in biological materials, particularly urine, by gas chromatography (GC) and high performance liquid chromatography (HPLC) (IARC 1981b; Jedrejczak and Gajnd 1993; Neumeister 1991; Nony and Bowman 1980; Nony et al. 1980). Most studies of the determination of benzidine in biological materials have concentrated on urine as a means of monitoring human exposure to benzidine in the workplace. The metabolites include monacetylated (Hodgson 1987), diacetylated, and conjugated metabolites. Of these, one that is commonly monitored is N,N'-diacetylbenzidine (N,N'-DAB). The analytical methods used to measure benzidine and its metabolites in biological materials are GC (NIOSH 1984b), gas chromatography/mass spectrometry (GC/MS) (Hsu et al. 1996; Hurst et al. 1981; Jedrejczak and Gajnd 1993), HPLC (IARC 1981b; Neumeister 1991), thin-layer chromatography (TLC) (NIOSH 1984a), and immunoassay (Johnson et al. 1981). Chemical derivatization is commonly employed in analytical methods based on GC analysis to enhance chromatography and detection (IARC 1981b; Hsu et al. 1996; Jedrejczak and Gajnd 1993; NIOSH 1984b).

Benzidine can be extracted from urine with a solvent such as chloroform or benzene, then re-extracted into aqueous HCl as the water-soluble cationic form. One of the reported methods (Jedrejczak and Gajnd 1993) converted all acetyl metabolites to benzidine via base hydrolysis prior to analysis to determine total benzidine. Benzidine and its metabolites can also be removed from urine by retention on solid adsorbents, followed by sample clean up and analysis (Hsu et al. 1996; Neumeister 1991).

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The technique of  $^{32}\text{P}$  post-labeling with HPLC analysis can be employed to detect and quantitate DNA-benzidine adducts formed *in vivo* in mice (Levy and Weber 1988). This method may be used for rapid screening to detect adduct formation and to measure differences in DNA damage caused by variations in metabolic pathways. Talaska et al. (1987) have also studied DNA-benzidine adducts in the livers of mice dosed with benzidine. After an intraperitoneal dose of 38 mg/kg, one adduct in 3.5 million normal nucleotides was detected.

Birner et al. (1990) have investigated adducts of benzidine and benzidine metabolites with hemoglobin. They identified adducts with benzidine, mono-N-acetylbenzidine, and 4-aminobiphenyl. It should be noted that the 4-aminobiphenyl-hemoglobin adduct is also commonly found in tobacco smokers (Hammond et al. 1993) and, thus, would not be specific for benzidine exposure or effect.

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

Thermospray techniques used in conjunction with mass spectrometry (MS) with or without high performance liquid chromatographic separation have proven useful for the determination of thermally labile compounds such as toxicant metabolites (Korfmacher et al. 1987) and should be applicable to the determination of benzidine in biological materials (Betowski et al. 1987). More recent liquid chromatography/MS interfaces, such as electrospray and particle beam, increase the potential for selective and sensitive benzidine determination.

Methods for the determination of benzidine in biological samples are summarized in Table 7-1.

## 7.2 ENVIRONMENTAL SAMPLES

Benzidine in environmental samples is most commonly determined by GC/MS (EPA 1982b) and HPLC (EPA 1982a). Methods using GC with Fourier transform infrared (GC/FT-IR) spectrophotometry have also been reported (EPA 1994). Although methods for the determination of benzidine in soils are reported, problems are so severe that Superfund has dropped benzidine from the analyte list in the current laboratory programs (Brumley and Brownrigg 1994).

**Table 7-1. Analytical Methods for Determining Benzidine in Biological Samples**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Acidification, addition of sodium nitrite and extraction with ether or acetone; destruction of excess nitrite and addition of 8-hydroxyquinoline; basification then extraction with 3-methyl-1-butanol	UV-Vis spectrophotometry	<30 µg/L (0.03ppm)	95%	Upadhyay and Gupta 1985
Urine (for benzidine and metabolites)	Extraction with benzene, heptafluorobutryl derivatization of non-conjugated compounds; base hydrolysis aqueous phase repeat extraction and derivatization for determination of conjugated metabolites	GC/ECD	1 µg/L (0.001ppm)	53 (7.9% RSD) at 0.01 ppm; 84 (6.0% RSD) at 0.1 ppm	IARC 1981b; Nony and Bowman 1980; Nony et al. 1980
Urine (for benzidine and metabolites)	Extraction with benzene and solvent exchange to methanol; hydrolysis of aqueous phase; extraction and solvent exchange	HPLC/UV	180 µg/L (0.18ppm)	64.7 at 0.5 ppm to 80.1 at 5 ppm (3% RSD)	IARC 1981b; Nony and Bowman 1980; Nony et al. 1980
Urine (total benzidine)	Base hydrolysis, addition of deuterated benzidine as internal standard, extraction with ethyl acetate/pyridine and derivatization with pentafluoropropionic anhydride	GC/MS (NICI)	0.5 µg/L (0.5 ppb)	75 at 10 µg/L; 87 at 100 µg/L (precision = 8.7% RSD at 10 µg/L)	Jedrejczak and Gajnd 1993

**Table 7-1. Analytical Methods for Determining Benzidine in Biological Samples (continued)**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Conjugation hydrolysis at pH=12 and isolation of free amine using C <sub>18</sub> SPE, elution with benzene	HPLC/UV, HPLC/EC	1.5 µg/L or 0.0015 ppm (UV); 0.24 µg/L or 0.24 ppb (EC)	>89	Neumeister 1991
Urine	Chloroform extraction, HCl extraction, derivatization with trinitrobenzene sulfonic acid	TLC/Spec (method 8304; screening test)	1 µg/L	70 at 5 µg/L	NIOSH 1984a
Urine (for benzidine and mono- and di-acetylbenzidine conjugates)	Base hydrolysis, extraction with benzene, derivatization with heptafluorobutyric anhydride, Florisil cleanup	GC/ECD (method 8306; quantitative method)	5 µg/L	92 (11% RSD at 10 µg/L)	NIOSH 1984b
Urine	No data	GC/MS	No data	No data	Hurst et al. 1981
Urine	Centrifugation of urine, pH adjustment to 6.8–7.2	RIA <sup>a</sup>	10 pg (low ppm given volume used)	92.7±0.8%	Johnson et al. 1981
Urine	Solid-phase extraction followed by successive washing and derivatization with pentafluoropropionic anhydride	GC/MS	0.5 µg/L (ppb)	87–89%	Hsu et al. 1996

<sup>a</sup>RIA is for diacetyl metabolite of benzidine.

EC = electrochemical detection; ECD = electron capture detector; GC = gas chromatography; HPLC = high performance liquid chromatography; MS = mass spectrometry; NICI = negative ion chemical ionization mass spectrometry; NIOSH = National Institute for Occupational Safety and Health; RIA = radioimmunoassay; SPE = solid phase extraction; TLC = thin-layer chromatography; UV = ultraviolet absorption spectrophotometry

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The GC/MS determination of benzidine in liquid and solid samples involves extraction into methylene chloride or chloroform followed by separation without derivatization on a gas chromatographic fused-silica capillary column coated with a slightly polar silicone material and detection with a mass spectrometer. Unfortunately, benzidine can be subject to oxidative losses during solvent extraction and concentration and its gas chromatographic separation properties are poor. Chromatographic problems can be overcome through the use of chemical derivatization; such is the case for benzidine in air where the pentafluoropropyl derivative is formed after extraction from the sorbent (Roussel et al. 1991). Benzidine and similar compounds can photodegrade (Brumley and Brownrigg 1994). Benzidine has been shown to be thermally-labile, and this could decrease the accuracy of GC-based methods (Riggin and Howard 1979). Sodium sulfate is commonly used to dry extracts prior to GC analysis and has been shown to adsorb benzidine (Riggin and Howard 1979); this can also impact the accuracy of the methods.

For the HPLC determination of benzidine in water, a relatively complicated procedure may be used (EPA 1982b) in which the analyte is extracted into chloroform, back-extracted with acid, neutralized, and extracted again with chloroform. The chloroform is exchanged to methanol and concentrated using a rotary evaporator and nitrogen blowdown, then brought to a 5-mL volume with an acetate buffer. Conditions are used that permit the separation of benzidine compounds by HPLC with electrochemical detection, which is now currently favored over spectrophotometric measurement (Trippel-Schulte et al. 1986). The method detection limit with HPLC separation and electrochemical detection is reported to be 0.08  $\mu\text{g/L}$ , and single-operator accuracy and precision for 30 analyses of 5 different types of water samples over a spike range of 1.0–50  $\mu\text{g/L}$  gave an average percent recovery of 65% and a standard deviation of 11.4% (EPA 1984). While this is an accepted method for benzidine analyses in water, some laboratories do not permit the use of chloroform and the electrochemical detector requires an operator with specialized skills (Hites and Budde 1991).

HPLC separation with ultraviolet (UV) absorption detection is used for the determination of benzidine in air (NIOSH 1985b). The analyte at levels in a range of 0.2–7  $\mu\text{g}$  per sample can be collected in a silica gel collection tube from up to 100 L of air. The estimated method limit of detection is 0.05  $\mu\text{g/sample}$ .

Methods for the determination of benzidine in environmental media are summarized in Table 7-2. While various methods have been employed to monitor exposures to benzidine in environmental materials, possible interferences may occur, reducing their sensitivity. Metal ions forming hydroxides in alkaline media may be expected to interfere in spectrophotometric analyses (Upadhyay and Gupta 1985). Also,

**Table 7-2. Analytical Methods for Determining Benzidine in Environmental Samples**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Glass fiber filter and silica gel; extraction with methanol containing triethylamine	HPLC	3 µg/m <sup>3</sup> (0.02 ppm)	70–97	IARC 1981a
Air	Silica gel sorption, desorption using methanol containing triethylamine	HPLC/UV (method 5509)	0.05 µg/sample 0.5–2.5 µg/m <sup>3</sup> or 0.004–0.0019 ppm, (depending on volume sampled)	No data	NIOSH 1985b
Air	Glass fiber filter impregnated with 5% sulfuric acid; release by sonication in acidic water; basification, extraction with toluene, and derivatization with pentafluoropropionic acid	GC/MS	1ng/m <sup>3</sup>	76	Roussel et al. 1991
Waste water	Addition of stable, isotope-labeled analogue of benzidine; basification, extraction with methylene chloride, solvent removal	GC/IDMS (method 1625)	50 µg/L (50 ppb)	Variable	EPA 1990b
Waste water	Extraction with chloroform, back-extraction with acid, neutralization and re-extraction with chloroform, exchange to methanol, solvent removal	HPLC/ELCD (method 605)	0.08 µg/L, depending on nature of interferences	0.70C + 0.06 where C = conc. in µg/L	EPA 1984
Waste water <sup>a</sup>	Extraction with methylene chloride at pH>11, solvent removal	GC/MS (method 625)	44 µg/L (44 ppb)	63–87	EPA 1982b

**Table 7-2. Analytical Methods for Determining Benzidine in Environmental Samples (continued)**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water, surface water, and waste water <sup>a</sup>	Extraction with methylene chloride followed by separation with reverse-phase HPLC	HPLC/PB/MS method 8325	No data	96–97	EPA 1996
Drinking water, surface water, and waste water <sup>a</sup>	Extraction with methylene chloride at pH>11, solvent removal	GC/MS (method 6410)	44 µg/L (44 ppb)	No data	APHA 1992
Water, soil, sediment	Water: pH adjustment to 8.5, extraction with methylene chloride, solvent removal and redissolution in mobile phase	CZE	Water: 0.1 mg/L (0.1 ppm)	Water: 82% at 1 g/L, 59% at 0.1 g/L	Brumley and Brownrigg 1994
	Soil, sediment: Soxhlet extraction, solvent removal and redissolution in mobile phase		Soil, sediment: approximately 1 mg/kg (1 ppm)	Soil: 56% at 0.9 g/kg	
Soil, sediment, solid waste <sup>a</sup>	Extraction with methylene chloride, solvent removal	GC/MS (method 8270D)	No data	No data	EPA 1998i
Food dyes	Reduction of bound amines with sodium dithionite, followed by chloroform extraction and derivatization with 2-naphthol-3,6-disulfonic acid, disodium salt	HPLC	3–4 ng/g	46%	Lancaster and Lawrence 1999

<sup>a</sup>Benzidine is subject to oxidative losses during extraction and evaporation.

APHA = American Public Health Association; CZE = capillary zone electrophoresis; ELCD = electrochemical detector; EPA = Environmental Protection Agency; FT-IR = Fourier transformed infrared spectrometry; GC = gas chromatography; HPLC = high performance liquid chromatography; IDMS = isotope dilution mass spectrometry; MS = mass spectrometry; NIOSH = National Institute for Occupational Safety and Health UV = ultraviolet absorption spectrophotometry

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certain amines can also interfere with this method at high concentrations. *p*-Nitroaniline and toluidine caused positive interferences (Upadhyay and Gupta 1985).

Studies designed to improve the analysis of semivolatile compounds will continue to yield refinements and improvements in the determination of benzidine in the environment. The current high level of activity in supercritical fluid extraction of solid and semi-solid samples should yield improved recoveries and sensitivities for the determination of benzidine in solid wastes, avoiding troublesome problems of analyte oxidation. The combination of SFE and supercritical fluid chromatography (SFC) has been described for the determination of sulfonylurea herbicides and their metabolites in complex matrices, including soil, plant materials, and a cell culture medium (McNally and Wheeler 1988). The approach described in this work should be applicable to many other toxicologically and environmentally significant analytes, including benzidine, and might well overcome the oxidation problems (EPA 1996) associated with the extraction and concentration of benzidine. Indeed, the ability to recover benzidine from spiked sand (Oostdyk et al. 1993a) and soil (Oostdyk et al. 1993b) is currently being investigated. In SFE, the addition of 1,6-hexanediamine in methanol to the extraction fluid was found to be needed to recover benzidine from spiked soil at the 79–82% range (Oostdyk et al. 1993b). The addition of the amine helps to displace benzidine from acidic surface sites on the soil. SFE has not been applied to soils containing many potentially interfering compounds. Benzidine should be amenable to SFC analysis, and this would reduce the need for chemical derivatization to improve chromatographic efficiency. Capillary zone electrophoresis (CZE) holds promise as a rapid determinative method that can provide for greater resolution and sensitivity than HPLC (Brumley and Brownrigg 1994). Sample preparation is still problematic. Immunoassay analysis (Vanderlaan et al. 1988) is an area of intense current activity from which substantial advances in the determination of benzidine in environmental samples can be anticipated.

The FDA has developed a method to quantify total benzidine (free and combined) in azo dyes that are currently approved for use in foods. The method involves reduction of the dye matrix with dithionite to free the combined benzidine, then extraction, diazotization, coupling with pyrazolone T, and analysis by HPLC with a photodiode array detector (Prival et al. 1993). The FDA has set a limit for free benzidine in these dyes at 1 ppb (see Chapter 8), but the detection limit for this method is reported as 5 ppb. This technique has been modified slightly, and a detection limit of 3–4 ng/g has been achieved (Lancaster and Lawrence 1999).



### 7.3 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 benzidine 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 benzidine.

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.

#### 7.3.1 Identification of Data Needs

**Methods for Determining Biomarkers of Exposure and Effect.** Methods are available for the determination of benzidine and/or its metabolites in urine (IARC 1981b; Hsu et al. 1996; Jedrejczak and Gajnd 1993; Neumiester 1991; NIOSH 1984a; Nony and Bowman 1980; Nony et al. 1980; Upadhyay and Gupta 1985). These permit the confirmation of human exposure to benzidine, its metabolites, and associated compounds such as the benzidine-based dyes. If an intake of 0.04 mg/kg/day is assumed, then a 70 kg person would ingest 2.59 mg/day. At this exposure, current analytical methods are sufficient to monitor resulting urinary concentrations at levels which may pose a human health threat. Reported methods for urine allow detection of benzidine and metabolites to below the  $\mu\text{g/L}$  concentration. Benzidine and its metabolites have a half-life in the body of only 5–6 hours (Neumeister 1991), so the methods are useful for detecting exposure for a short period after the exposure has occurred. More sensitive methods would be required if there is a need to increase the time from exposure termination to the collection of a sample containing measurable benzidine.

Adducts of benzidine and benzidine metabolites have been found in DNA (Levy and Weber 1988; Talaska et al. 1987) and hemoglobin (Birner et al. 1990; Hammond et al. 1993). In addition to hemoglobin adducts of the N-acetylated benzidines, an adduct with 4-aminobiphenyl has been measured (Birner et al. 1990). As noted above, 4-aminobiphenyl-hemoglobin adduct is also commonly found in

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tobacco smokers (Hammond et al. 1993) so the presence of this adduct would not be specific for benzidine exposure or effect. Additional information is needed regarding the quantity of adduct formed after a given exposure and how the quantities of adduct relate to effect. It is likely that more sensitive and specific methods for adducts are needed to properly identify and quantify the exposures and potential effects.

**Methods for Determining Parent Compounds and Degradation Products in Environmental**

**Media.** Standard methods are available for the determination of benzidine in environmental samples including air, water, soil, sediment, and solid waste (APHA 1992; EPA 1982b, 1984, 1990b, 1996, 1998i; NIOSH 1985b). Assuming an oral exposure of 0.04 mg/kg/day, analytical methods for benzidine in drinking water are sufficient (2 L consumed per day by a 70 kg person). Problems need to be solved regarding the oxidation of benzidine during extraction from environmental samples and subsequent concentration. This is especially true if the quantitation of benzidine in soils is required. More sensitive methods are needed in order to accurately measure benzidine levels in soils and sediment. This is particularly true where benzidine may be present in significant quantities, such as at hazardous waste sites. It should be noted that background levels of benzidine are expected to be very low in most environmental media, and may be below the detection limits regardless of the sensitivity of the technique employed. No MRL has been established for inhalation exposures but many of the analytical methods for benzidine in air are very sensitive (e.g., Roussel et al. 1991; 1 ng/m<sup>3</sup>; and NIOSH 1985b; 0.5 µg/m<sup>3</sup>), so it is not likely that new methods would be required. If MRLs are established for inhalation exposure, the values will dictate whether additional methods are needed.

**7.3.2 Ongoing Studies**

No information could be identified on ongoing studies related directly to the development of analytical techniques used to quantify benzidine in biological or environmental media.

## 8. REGULATIONS AND ADVISORIES

International, national, and state regulations and advisories regarding benzidine in air, water, and other media are summarized in Table 8-1. These values have been established because of benzidine's potential to cause adverse health effects in exposed people.

The EPA (IRIS 2001) has calculated a chronic oral Reference Dose (RfD) for benzidine of  $3 \times 10^{-3}$  mg/kg/day based on a LOAEL of 2.7 mg/kg/day for brain cell vacuolization and liver cell alterations observed in female mice exposed for their lifetimes to 20 ppm benzidine dihydrochloride in the drinking water (Littlefield et al. 1983). A NOAEL was not established. The LOAEL of 2.7 mg/kg/day benzidine was divided by uncertainty factors of 10 for extrapolation from animals to humans, 10 for human variability in sensitivity, and 10 for the uncertainty in estimating a NOAEL from a LOAEL. The EPA (IRIS 2001) has derived an inhalation cancer slope factor of  $2.3 \times 10^2$  (mg/kg/day)<sup>-1</sup> based on the incidence of bladder cancer in workers exposed to benzidine as reported by Zavon et al. (1973). An oral slope factor of  $2.3 \times 10^2$  (mg/kg/day)<sup>-1</sup> was also estimated from the inhalation exposure data (IRIS 2001).

Benzidine is on the list of chemicals appearing in "The Emergency Planning and Community Right-to-Know Act of 1986" (EPCRA) (EPA 1988a). Section 313 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.

OSHA standards strictly regulate the use of a solid or liquid mixture containing less than 0.1 percent by weight or volume of benzidine (29CFR 1910.1003). No permissible exposure limit (PEL) is established.

Under the Resource Conservation and Recovery Act (RCRA), benzidine is listed as a hazardous waste when it is a discarded commercial product, off-specification species, container residue, or spill residue thereof (EPA 1980b).

## 8. REGULATIONS AND ADVISORIES

**Table 8-1. Regulations and Guidelines Applicable to Benzidine**

Agency	Description	Information	References
<u>INTERNATIONAL</u>			
Guidelines:			
IARC	Carcinogenicity classification	Group 1 <sup>a</sup>	IARC 2001
<u>NATIONAL</u>			
Regulations and Guidelines:			
a. Air			
ACGIH	Carcinogenicity classification <sup>b</sup>	A1 <sup>c</sup>	ACGIH 2000
EPA	National emission standards for HAP's for source categories—demonstration of early reduction of high-risk pollutants		EPA 2001e 40CFR63.74
NIOSH	REL not established	potential carcinogen	NIOSH 1999
OSHA	PEL not established	carcinogen	OSHA 2001 29CFR1910.1003
USC	HAP		USC 2001 42USC7412
b. Water			
EPA	Electroplating point source category	$>1 \times 10^{-2}$ mg/L	EPA 2000a 40CFR413.02(i)
	Metal finishing point source category	$>1 \times 10^{-2}$ mg/L	EPA 2000b 40CFR433.11(e)
	Toxic pollutant effluent standards		EPA 2000c 40CFR129.104
	Ambient water criterion	0.1 µg/L	
	Benzidine manufacturer calculated over 1-month monthly average daily loading sample representing any work day	10 µg/L 0.130 kg/kkg 50 µg/L	
	Benzidine based-dye applicators calculated over 1-month sample representing any work day	10 µg/L 25 µg/L	

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**Table 8-1. Regulations and Guidelines Applicable to Benzidine (continued)**

Agency	Description	Information	References
<u>NATIONAL (cont.)</u>			
EPA	Toxic pollutant effluent standards—regulated toxic pollutant		EPA 2001h 40CFR129.4
	Water quality criteria for human health for consumption of:		EPA 1999a
	Water and organism	1.2x10 <sup>-4</sup> µg/L <sup>d</sup>	
	Organism only	5.4x10 <sup>-4</sup> µg/L <sup>d</sup>	
c. Food			
FDA	Limit in color additives for foods FD&C Yellow No. 5	# 1ppb	FDA 2000a 21CFR74.705
	Limit in color additives for foods FD&C Yellow No. 6	# 1ppb	FDA 2000b 21CFR74.706
d. Other			
DOT	Reportable quantity	1 pound	DOT 2001 49CFR172.101 Appendix A
EPA	Carcinogenicity classification	Group A <sup>e</sup>	EPA 2001a
	Cancer slope factor (inhalation and oral)	2.3x10 <sup>2</sup> (mg/kg/day) <sup>-1</sup>	
	Carcinogenic inhalation unit risk	6.7x10 <sup>-2</sup> (µg/m <sup>3</sup> ) <sup>-1</sup>	IRIS 2001
	Carcinogenic drinking water unit risk	6.7x10 <sup>-3</sup> (µg/L) <sup>-1</sup>	
	CERCLA—toxic pollutant	Subject to section 307(a) of CERCLA	EPA 2000d 40CFR401.15
	Community Right-to-Know; toxic chemical release reporting—effective date	01/01/87	EPA 1999c 40CFR372.65
	Health and environmental protection standards at uranium and thorium tail millings—listed constituent		EPA 2001b 40CFR192 Appendix I
	Health based limits for exclusion of waste-derived residues—concentration limit for residue	1x10 <sup>-6</sup> mg/kg	EPA 2001c 40CFR266 Appendix VII

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**Table 8-1. Regulations and Guidelines Applicable to Benzidine (continued)**

Agency	Description	Information	References
<u>NATIONAL (cont.)</u>			
EPA	Identification and listing of hazardous waste—discarded commercial chemical containers, off-specification species, container residues, and spill residues	U021	EPA 2000e 40CFR261.33
	Identification and listing of hazardous waste—comparable/Syngas fuel exclusion		EPA 2001d 40CFR261.38
	Minimum required detection limit	2,400 mg/kg	
	Reportable quantity	1 pound	EPA 1999b 40CFR302.4
	RfD	$3 \times 10^{-3}$ mg/kg/day	IRIS 2001
	Risk specific doses		EPA 2001f 40CFR266
	Unit risk	$6.7 \times 10^{-2}$ µg/L	Appendix V
	RsD	$1.5 \times 10^{-4}$ µg/L	
	Steam electric power generating point source category—priority pollutants		EPA 2001g 40CFR423 Appendix A
	TSCA—health and safety data reporting		EPA 2001i 40CFR716.120
	Effective date	06/01/87	
	Sunset date	06/01/87	
	TSCA—significant new uses of chemical substances		EPA 2001j 40CFR721.1660
	TSD facilities—compounds with a Henry's law constant	less than 0.1 Y/K	EPA 2001k 40CFR265 Appendix VI
<u>STATE</u>			
Regulations and Guidelines:			
a. Air			
Georgia	Instream concentration	$5.35 \times 10^{-4}$ µg/L	GDNR 2000
Kansas	Ambient air quality standard	$3 \times 10^{-4}$ tons/year	CDC 1999
b. Water			
Arizona	Drinking water guideline	$1 \times 10^{-4}$ µg/L	HSDB 2001

## 8. REGULATIONS AND ADVISORIES

**Table 8-1. Regulations and Guidelines Applicable to Benzidine (continued)**

Agency	Description	Information	References
<u>STATE (cont.)</u>			
Florida	Drinking water guideline	250 µg/L	HSDB 2001
New Hampshire	Drinking water guideline	2x10 <sup>-4</sup> µg/L	HSDB 2001
c. Food		No data	
d. Other		No data	

<sup>a</sup>Group A1: sufficient evidence for carcinogenicity in animals and humans

<sup>b</sup>Skin notation: danger of cutaneous absorption

<sup>c</sup>Group A: human carcinogen

<sup>d</sup>This criterion is based on carcinogenicity of 10<sup>-6</sup> risk. Alternate risk levels may be obtained by moving the decimal point (e.g., for a risk level of 10<sup>-5</sup>, move the decimal point in the recommended criterion one place to the right).

<sup>e</sup>Group A: human carcinogen

ACGIH = American Conference of governmental Industrial Hygienists; CDC = Center for Disease Control; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DOT = Department of Transportation; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; GDNR = Georgia Department of Natural Resources; HAP = hazardous air pollutant; HSDB = Hazardous Substances Data Bank; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; NIOSH = National Institute of Occupational Safety and Health; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; REL = recommended exposure limit; RfD = oral reference dose; RsD = risk specific dose; TSCA = Toxic Substances Control Act; TSD = transport, storage, and disposal; USC = United States Code





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## 10. 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 ( $K_d$ )**—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)**—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**—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**—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.

**Biota**—All living organisms that exist in an area.

**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.

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**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.

**FEFR<sub>25-75</sub>**—Forced expiratory flowrate between 25 and 75%.

**FEV<sub>1.0</sub>**—Forced expiratory volume in 1.0 seconds.

**FVC**—Forced vital capacity.

**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.



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

**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**—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 Concentration<sub>(LO)</sub> (LC<sub>LO</sub>)**—The lowest concentration of a chemical in air which has been reported to have caused death in humans or animals.

**Lethal Concentration<sub>(50)</sub> (LC<sub>50</sub>)**—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<sub>(LO)</sub> (LD<sub>LO</sub>)**—The lowest dose of a chemical introduced by a route other than inhalation that is expected to have caused death in humans or animals.

**Lethal Dose<sub>(50)</sub> (LD<sub>50</sub>)**—The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

**Lethal Time<sub>(50)</sub> (LT<sub>50</sub>)**—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

**Lowest-Observed-Adverse-Effect Level (LOAEL)**—The lowest dose 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.

**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.

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**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 genetic material in a body cell. 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 chemical.

**No-Observed-Adverse-Effect Level (NOAEL)**—The dose of 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 (OR)**—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 8-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**—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.

**Pharmacokinetic Model**—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**—A type of physiologically-based dose-response 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

## 10. GLOSSARY

describe the biological effect (response) produced by the system following exposure to an exogenous substance.

**Physiologically Based Pharmacokinetic (PBPK) Model**—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 such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

**ppbv**—Parts per billion by volume.

**ppmv**—Parts per million by volume.

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

**Proportionate Mortality Ratio (PMR)**—The ratio of a cause-specific mortality proportion in an exposed group to the mortality proportion in an unexposed group; mortality proportions may be adjusted for confounding variables such as age. Cause-specific mortality proportions can be calculated when the cohort (the population at risk) cannot be defined due to inadequate records, but the number of deaths and the causes of deaths are known.

**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  $\mu\text{g/L}$  for water,  $\text{mg/kg/day}$  for food, and  $\mu\text{g/m}^3$  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  $\text{mg/m}^3$  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.

**Relative Risk (RR)**—The risk expressed as a ratio of the incidence of diseased subjects exposed to a particular risk factor to the incidence of diseased subjects in a non-exposed referent group.

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**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.

**Standardized Mortality Ratio (SMR)**—The ratio of a cause-specific mortality rate in an exposed cohort during a given period to the mortality rate of an unexposed cohort; mortality rates are often adjusted for age or other confounding variables.

**Standardized Proportionate Incidence Ratio (SPIR)**—Similar to a Proportionate Mortality Ratio (PMR) in that it is a ratio of a proportion of a specific disease in an exposed group compared with the proportion in an unexposed group.

**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).

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**Time-Weighted Average (TWA)**—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

**Toxic Dose<sub>(50)</sub> (TD<sub>50</sub>)**—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.

**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 UF 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 10 and 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. 99–499], 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 (1–14 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.

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

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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 agency wide 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 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

##### Relevance to Public Health

This chapter 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 chapter 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 chapter. 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 Chapter 3 Data Needs section.

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**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, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "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).

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, 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 end point 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.

**Chapter 3****Health Effects****Tables and Figures for Levels of Significant Exposure (LSE)**

Tables (3-1, 3-2, and 3-3) and figures (3-1 and 3-2) 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).

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The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 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 3-1**

- (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 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not therefore have all five of the tables and figures.
- (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 3-1).
- (5) Species The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "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.

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- (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 end point 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 9 of the profile.
- (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 3-1**

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<sup>3</sup> or ppm and oral exposure is reported in mg/kg/day.
- (16) NOAEL In this example, 18r NOAEL is the critical end point 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.

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- (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 6

**Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

Key to figure <sup>a</sup>	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
<b>INTERMEDIATE EXPOSURE</b>							
	5	6	7	8	9		10
3 6	Systemic	9	9	9	9		9
4 6	18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3 <sup>b</sup>	10 (hyperplasia)	Nitschke et al. 1981
<hr style="border-top: 1px dashed black;"/>							
<b>CHRONIC EXPOSURE</b>							
						11	
	Cancer					9	
38	Rat	18 mo 5 d/wk 7 hr/d				20	(CEL, multiple organs) Wong et al. 1982
39	Rat	89–104 wk 5 d/wk 6 hr/d				10	(CEL, lung tumors, nasal tumors) NTP 1982
40	Mouse	79–103 wk 5 d/wk 6 hr/d				10	(CEL, lung tumors, hemangiosarcomas) NTP 1982

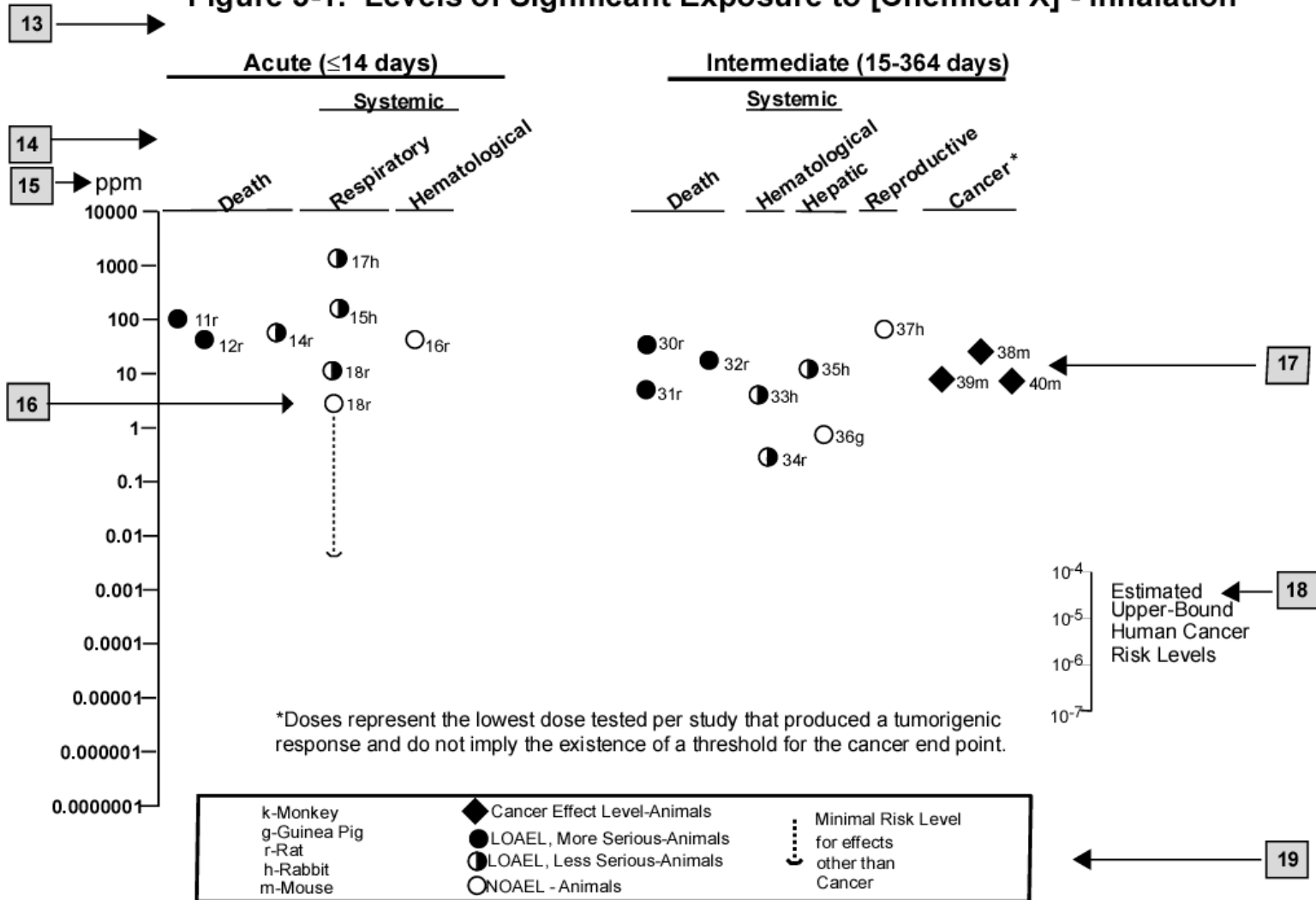
12 6

<sup>a</sup> The number corresponds to entries in Figure 3-1.

<sup>b</sup> Used to derive an intermediate inhalation Minimal Risk Level (MRL) of  $5 \times 10^{-3}$  ppm; 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**

**Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation**



\*\*\*DRAFT -- DO NOT CITE OR QUOTE -- October 4, 2001\*\*\*

Version 1.0





## 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
ECD	electron capture detection
ECG/EKG	electrocardiogram

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EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F <sub>1</sub>	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	<i>Federal Register</i>
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
K <sub>d</sub>	adsorption ratio
kg	kilogram
kgg	metric ton
K <sub>oc</sub>	organic carbon partition coefficient
K <sub>ow</sub>	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC <sub>Lo</sub>	lethal concentration, low
LC <sub>50</sub>	lethal concentration, 50% kill
LD <sub>Lo</sub>	lethal dose, low
LD <sub>50</sub>	lethal dose, 50% kill
LT <sub>50</sub>	lethal time, 50% kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter
MA	<i>trans,trans</i> -muconic acid
MAL	Maximum Allowable Level
mCi	millicurie
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
mg	milligram
min	minute
mL	milliliter

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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

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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 <sub>50</sub>	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
>	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

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$\mu\text{g}$	microgram
$q_1^*$	cancer slope factor
-	negative
+	positive
(+)	weakly positive result
(-)	weakly negative result



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