# TOXICOLOGICAL PROFILE FOR 1,3-DINITROBENZENE AND 1,3,5-TRINITROBENZENE

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

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## 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 ATSDR and the Environmental Protection Agency (EPA) and in support of Department of Defense information needs. 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 being described. Each profile identifies and reviews the key literature (that has been peer-reviewed) that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

Each toxicological profile begins with a public health statement, which describes in nontechnical language a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, when 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 significant to protect public health will be identified by ATSDR and the EPA. The focus of the profiles is on health and toxicologic information; therefore, we have included this information in the beginning of the document.

Each profile must include the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects.

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects.

(C) When appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that might 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.

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). Section 211 of SARA also amended Title 10 of the U. S. Code, creating the Defense Environmental Restoration Program. Section 2704(a) of Title 10 of the U. S. Code directs the Secretary of Defense to notify the Secretary of Health and Human Services of not less than 25 of the most commonly found unregulated hazardous substances at defense facilities.

Section 2704(b) of Title 10 of the U. S. Code directs the Administrator of the Agency for Toxic Substances and Disease Registry (ATSDR) to prepare a toxicological profile for each substance on the list provided by the Secretary of Defense under subsection (b).

***Foreword***

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

David Satcher, M.D., Ph.D.
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## THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Green Border Review. Green Border review assures consistency with ATSDR policy.

2. 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.

3. 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.

4. Quality Assurance Review. The Quality Assurance Branch assures that consistency across profiles is maintained, identifies any significant problems in format or content, and establishes that Guidance has been followed.

## PEER REVIEW

A peer review panel was assembled for 1,3-DNB and 1,3,5-TNB. The panel consisted of the following members:

1. Dr. Gordon Edwards, President, Toxicon Associates, Natick, Massachusetts;

2. Dr. William George, Professor, Department of Pharmacology, Tulane University, New Orleans, Louisiana; and

3. Dr. Lloyd Hastings, Research Associate* Professor, Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio.

These experts collectively have knowledge of 1,3-DNB's and 1,3,5-TNB'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 statement was prepared to give you information about 1,3-dinitrobenzene (1,3-DNB) and 1,3,5-trinitrobenzene (1,3,5-TNB) and to emphasize the human health effects that may result from exposure to them. The Environmental Protection Agency (EPA) has identified 1,397 waste sites as the most serious in the nation. These sites make up the National Priorities List (NPL) and are the sites targeted for long-term federal clean-up activities. 1,3-DNB and 1,3,5-TNB have been found in at least 19 of the sites on the NPL. However, the number of NPL sites evaluated for 1,3-DNB and 1,3,5-TNB is not known. As EPA evaluates more sites, the number of sites at which 1,3-DNB and 1,3,5-TNB are found may increase. This information is important because exposure to 1,3-DNB and 1,3,5-TNB may cause harmful health effects and because these sites are potential or actual sources of human exposure to 1,3-DNB and 1,3,5-TNB.

When a chemical 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 as a chemical emission. This emission, which is also called a release, does not always lead to exposure. You can be exposed to a chemical only when you come into contact with the chemical. You may be exposed to it in the environment by breathing, eating, or drinking substances containing the chemical or from skin contact with it.

If you are exposed to a hazardous chemical such as 1,3-DNB or 1,3,5-TNB, several factors will determine whether harmful health effects will occur and what the type and severity of those health effects will be. These factors include the dose (how much), the duration (how long), the route or pathway by which you are exposed (breathing, eating, drinking, or skin contact), the other chemicals to which you are exposed, and your individual characteristics such as age, sex, nutritional status, family traits, lifestyle, and state of health.

## 1.1 WHAT ARE 1,3-DNB and 1,3,5-TNB?

1,3-DNB and 1,3,5-TNB are synthetic substances that are used in explosives. In making 1,3,5-TNB, 1,3-DNB is often made first. Both 1,3-DNB and 1,3,5-TNB are formed as by-products when another explosive, trinitrotoluene (TNT), is made. 1,3-DNB is also used to make certain dyes, as an intermediate in the synthesis of .organic chemicals, and in the plastics manufacturing industry. 1,3,5-TNB is used in .making rubber. Other names for 1,3-DNB include *m*-dinitrobenzene, 1,3-dinitrobenzol, 2,4-dinitrobenzene, binitrobenzene, and *m*-DNB. Other names for 1,3,5-TNB include benzite, s-trinitrobenzene, sy*m*-trinitrobenzene, symmetric trinitrobenzene, syn-trinitrobenzene, and TNB. Both 1,3-DNB and 1,3,5-TNB are yellow, crystal-like solids at room temperature. They may exist in the air in very small amounts as a dust or a vapor and can dissolve in certain liquids. If either compound is put under very high heat, it will explode. These compounds have no odor or taste.

In this profile, 1,3-DNB and 1,3,5-TNB are discussed together because they have very similar structures. Since the two compounds are similar in structure, their toxic effects may not be very different. More information on the chemical and physical properties of 1,3-DNB and 1,3,5-TNB is found in Chapter 3. More information on the production and use of 1,3-DNB and 1,3,5-TNB is in Chapter 4.

## 1.2 WHAT HAPPENS TO 1,3-DNB or 1,3,5-TNB WHEN IT ENTERS THE ENVIRONMENT?

Waste discharges from Army ammunition plants or other chemical manufacturers are the primary sources for the releases of both compounds to air, water, and soil. They can also enter the environment from their use as explosives and from spills or improper disposal. It is unlikely that either compound would normally be.found in the air. However, under some industrial use conditions, some 1,3-DNB and 1,3,5-TNB may enter the air in the form of dust. We have very little information about what happens to 1,3-DNB and 1,3,5-TNB in the air. The small amounts of 1,3,5-TNB that may enter the air are likely to break down very slowly. It might persist for many years in the air. 1,3-DNB is also likely to break down in the air;

however, we do not know how long this would take. Both compounds are slightly soluble in water. 1,3-DNB evaporates slowly from water; 1,3,5-TNB does not evaporate from water. Neither compound sticks strongly to soil; therefore, both can move through soil into groundwater. I,3-DNB breaks down slowly in water and soil. It stays for days to months in water. Although 1,3,5-TNB probably breaks down in water and soil, we do not know how long this takes. Neither compound is likely to build up in fish or humans. See Chapters 4 and 5 for more information on 1,3-DNB and 1,3,5-TNB in the environment.

## 1.3 HOW MIGHT I BE EXPOSED TO 1,3-DNB OR 1,3,5-TNB?

Most of the population will not be exposed to 1,3-DNB or 1,3,5-TNB. If you live or work near an Army ammunition plant or other chemical manufacturer, you may be exposed to these compounds by contaminated drinking water, food, air, or soil. At this time, it is not known how much of these compounds you might consume or how much might be in the air. We also do not know how many workers are exposed to the compounds. Both 1,3-DNB and 1,3,5-TNB have been found in water and soil at some Army ammunition plants. Groundwater samples had levels of 1,3-DNB ranging from 0.0012 to 0.195 parts per one million parts of water (ppm). 1,3-DNB was present at higher levels in soil, with concentrations ranging from 0.77 to 1.5 ppm. 1,3,5-TNB was also present in groundwater samples at concentrations up to 8 ppm. 1,3,5-TNB was present at higher levels in soil, with concentrations ranging from 368 to 3,920 ppm. See Chapter 5 for more information on exposure to 1,3-DNB and 1,3,5-TNB.

## 1.4 HOW CAN 1,3-DNB AND 1,3,5-TNB ENTER AND LEAVE MY BODY?

1,3-DNB can enter your bloodstream if you breathe it in the air or get it on your skin. There is no information on how 1,3,5-TNB can enter or exit your body. Exposure of the general population to 1,3-DNB or 1,3,5-TNB is not likely, so most people exposed to 1,3-DNB have come in contact with it in their work place. Results of studies in people and animals show that 1,3-DNB enters the body very quickly through the skin or lungs. Once 1,3-DNB is inside your body, it breaks down quickly. 1,3-DNB and its related breakdown products also exit the body very quickly in the urine. Some breakdown products of 1,3-DNB may also

leave in the feces. Results of studies in people and animals show that most of the 1,3-DNB exits the body within 2 to 3 weeks after exposure. Chapter 2 has more information on how 1,3-DNB can enter and leave the body.

## 1.5 HOW CAN 1,3-DNB AND 1,3,5-TNB AFFECT MY HEALTH?

1,3-DNB and 1,3,5-TNB are suspected to cause similar health effects. Exposure to high concentrations of 1,3-DNB can reduce the ability of blood to carry oxygen and can cause your skin to become bluish in color. If you are exposed to 1,3-DNB for a long time, you can develop a reduction (or loss) in the number of red blood cells (anemia). Other symptoms of 1,3-DNB exposure include headache, nausea, and dizziness. We do not know if there are any long-term health effects of exposure to 1,3-DNB or 1,3,5-TNB in people. We also do not know if 1,3-DNB or 1,3,5-TNB causes birth defects or cancer in people.

Results of studies in animals show that *effects* of 1,3-DNB and 1,3,5-TNB *on* the blood are similar to the effects seen in people. Results from animal studies also show some other effects of 1,3-DNB exposure, such as behavioral changes, damaged sperm production, and male reproductive damage. We do not know if these other effects could occur in people. Animal studies also show that, in certain cases, a large enough single oral dose of 1,3-DNB can cause death. Neither 1,3-DNB or 1,3,5-TNB have been tested to see whether or not they cause cancer in animals.

The Environmental Protection Agency has determined that 1,3-DNB is not classifiable as to its human carcinogenicity and has not classified the carcinogenicity of 1,3,5-TNB.

More information on the health effects of 1,3-DNB and 1,3,5-TNB is in Chapter 2.

## 1.6 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO 1,3-DNB OR 1,3,5-TNB?

No tests are available commercially to determine if you have been exposed to 1,3-DNB or 1,3,5-TNB. There are tests to detect 1,3-DNB and its breakdown products in the blood and urine of exposed animals, but these tests have not been used for people. Refer to Chapters 2 and 6 for more information on these tests for animals.

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

The government has developed regulations and guidelines for 1,3-DNB and 1,3,5-TNB. These are designed to protect the public from the harmful health effects of the chemicals. EPA has classified 1,3-DNB and 1,3,5-TNB as hazardous wastes that must meet certain disposal requirements. The Department of Transportation has many regulations on the transportation of explosives including 1,3-DNB and 1,3,5-TNB.

The Occupational Safety and Health Administration (OSHA) regulates levels of 1,3-DNB in the workplace. The maximum allowable amount of 1,3-DNB in workroom air during an 8-hour workday, 40-hour workweek, is 1.0 milligram per cubic meter ($mg/m^3$).

See Chapter 7 for more information on regulations and guidelines on 1,3-DNB and 1,3,5-TNB.

## 1.8 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, Georgia 30333
(404) 639-6000

This agency can also tell you where to find the nearest occupational and environmental health clinic. These clinics specialize in the recognition, evaluation, and treatment of illness resulting from exposure to hazardous substances.

# 2. HEALTH EFFECTS

## 2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of 1,3-dinitrobenzene and 1,3,Minitrobenzene (1,3-DNB and 1,3,5-TNB) and a depiction of significant exposure levels associated with various adverse health effects. It contains descriptions and evaluations of studies and presents levels of significant exposure for 1,3-DNB and 1,3,5-TNB based on toxicological studies and epidemiological investigations.

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

## 2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals 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 and lymphoreticular, 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 lowestobserved-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 in not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify

these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between “less serous” 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 to determine 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 tables and figures may differ depending on the user’s perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons may be interested in levels of exposure associated with “serious” effects. Public health officials and project managers concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAEL) 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.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for 1,3-DNB and 1,3,5-TNB. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. 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 within a given route of exposure. MRLs are based on noncancer health effects only and do not reflect a consideration of carcinogenic effects.. Marls can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990b), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix A). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

### 2.2.1 lnhalation Exposure

1,3-DNB and 1,3,5-TNB are nitrobenzene compounds that are structurally similar. The only difference in structure between 1,3-DNB and 1,3,5-TNB is the presence of an additional nitro group in 1,3,5-TNB. There is no information on 1,3,5-TNB exposure by the inhalation route. There is also very little information regarding inhalation exposure to 1,3-DNB.

The one study found on inhalation exposure is a report of an occupational exposure to 1,3-DNB (Okuba and Shigeta 1982). The study indicates that inhalation was the main route of exposure, while skin absorption was much less important. Since precise labels of exposure are not known, the results are not presented in a table or figure.

#### 2.2.1.1 Death

No studies were located regarding lethal effects in humans or animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

#### 2.2.1.2. Systemic Effects

No studies were located regarding gastrointestinal, musculoskeletal, dermal, or ocular effects in humans or animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

**Respiratory Effects.** No studies were located regarding respiratory effects in humans after inhalation exposure to 1,3,5-TNB. One retrospective study (Okuba and Shigeta 1982) of acute occupational exposure to 1,3-DNB dust particles was located. Six workers were removing crystallized 1,3-DNB from tank and were protected with gauze masks and rubber gloves. Exposure occurred over a period of 6 days. By the end of the exposure period, some of the workers complained of slight dyspnea upon exertion. Inhalation was considered to be a primary route of exposure because a relatively small skin area (face and neck) was exposed. Limitations of this study include lack of

information on the concentration of 1,3-DNB in the air, the amount of particulate 1,3-DNB deposited on workers' skin, and the exact duration of exposure.

No studies were located regarding respiratory effects in animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

**Cardiovascular Effects.** No studies were located regarding cardiovascular effects in humans after inhalation exposure to 1,3,5-TNB. One retrospective study (Okubo and Shigeta 1982) of acute occupational exposure to 1,3-DNB dust particles was located. Six workers were removing crystallized 1,3-DNB from a tank and were protected with gauze masks and rubber gloves. Exposure occurred over a period of 6 days. By the end of the exposure period, some of the workers complained of palpitations upon exertion. Inhalation was considered to be a primary route of exposure because a relatively small skin area (face and neck) was exposed. Limitations of this study include lack of information on the concentration of 1,3-DNB in the air, the amount of particulate 1,3-DNB deposited on workers' skin, and the exact duration of exposure.

**Hematological Effects.** No studies were located regarding hematological effects in humans after inhalation exposure to 1,3,5-TNB. Cyanosis was the first symptom noticeable within a day after an acute human exposure to I,3-DNB. Slight-to-moderate anemia with a decrease in specific gravity of the whole blood was also observed in all six workers engaged in the clean-up of crystallized 1,3-DNB (Okubo and Shigeta 1982). It should be noted however that measurement of specific gravity of blood is not common practice and represents an indirect measurement of anemia. No information on methemoglobin levels was available. It is important to stress that pathological effects (cyanosis due to methemoglobin formation) observed after exposure to 1,3-DNB in one system can be manifested as symptoms in another. Other limitations of this study include lack of information on 1,3-DNB concentration in the air and the fact that data were collected 10 days after exposure. No long-term adverse effects were noted in any of the workers that were followed for up to 10 years after exposure to 1,3-DNB.

No studies were located regarding hematological effects in animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

**Hepatic Effects.** No studies were located regarding hepatic effects in humans after inhalation exposure to 1,3,5-TNB. Data on the effects of 1,3-DNB on the liver are inconclusive. Serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels in humans were within normal limits after a single acute exposure to 1,3-DNB (Okubo and Shigeta 1982). Bilirubin was found in the urine of all workers who were strongly positive for urobilinogen indicating an unspecified degree of hepatobiliary disease. This study is limited by the fact that there are no data on the concentration of 1,3-DNB in the air.

No studies were located regarding hepatic effects in animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

**Renal Effects.** No studies were located regarding renal effects in humans after inhalation exposure to 1,3,5-TNB. Elevated levels of urobilinogen were found in all workers exposed to 1,3-DNB indicating hemolysis (Olcubo and Shigeta 1982). Limitations of the study are that data were collected 10 days after exposure and information is lacking on the dose of 1,3-DNB.

No studies were located regarding renal effects in animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

#### 2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

#### 2.2.1.4 Neurological Effects

No studies were located regarding neurological effects in humans after inhalation exposure to 1,3,5-TNB. Limited information is available regarding the neurological effects of 1,3-DNB. Slight headache, nausea, dizziness, and fatigue were the symptoms reported in workers after inhalation exposure to 1,3-DNB (Okubo and Shigeta 1982). These symptoms diminished gradually, and the recovery period was different in each worker. No long-term effects due to inhalation exposure to 1,3-DNB were found in any of the workers for up to 10 years after exposure.

No studies were located regarding neurological effects in animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

No studies were located regarding the following health effects in humans or animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB:

**2.2.1.5 Reproductive Effects**

**2.2.1.6 Developmental Effects**

**2.2.1.7 Genotoxic Effects**

Genotoxiciey studies are discussed in Section 2.4.

**2.2.1.8 Cancer**

No studies were located regarding cancer effects in humans or animals after inhalation exposure to 1,3-DNB or 1,3,5-TNB.

**2.2.2 Oral Exposure**

**2.2.2.1 Death**

No studies were located regarding death in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

The oral $LD_{50}$ values for 1,3,5-TNB and 1,3-DNB in rats were 275 mg/kg and 59 mg/kg, respectively (Desai et al. 1991); no further details were provided in that abstract. The oral $LD_{50}$ value for 1,3-DNB in adult male and female Carworth Farms rats was 91 mg/kg and 81 mg/kg, respectively (Cody et al. 1981). Increased mortality in Sprague-Dawley rats (Linder et al. 1990) and rabbits (Parke 1961) was observed at 48 and 100 mg/kg/day, respectively. The age of the animals appeared to influence the acute toxicity of 1,3-DNB, the older animals appearing more susceptible to general toxicity than younger animals (Linder et al. 1990). Increased mortality was observed in prepubertal mice treated with 40 mg/kg/day (Evenson et al. 1989a). Decreased survival in animals consuming 1,3-DNB over longer periods was seen at lower doses in Sprague-Dawley rats. Rats exposed to

1,3-DNB for 11 weeks exhibited an increase in mortality at 6 mg/kg/day (Linder et al. 1986). At slightly a higher doses of 1,3-DNB (12-14 mg/kg/day), increased mortality in rats (Carworth Farms) occurred between weeks 4 and 7 (Cody et al. 1981). These results indicate that there may be a difference in susceptibility to 1,3-DNB toxicity associated with the age of animals.

The $LD_{50}$ value and all reliable LOAEL values for death in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

#### 2.2.2.2 Systemic Effects

The highest NOAEL value and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

**Respiratory Effects.** The only information regarding respiratory effects of 1,3-DNB or 1,3,5-TNB in humans is that of a man who swallowed 30-40 mL of a varnish containing a nitrobenzene dye (Kumar et al. 1990). Upon admission to the hospital he was comatose and his breathing was described as very shallow. This breathing pattern may have been indirectly caused by a significant increase in methemoglobin.

Data in animals are limited to a report in which no histopathologic alterations were seen in the lungs of rats given up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water (Cody et al. 1981).

**Cardiovascular Effects.** The only information available regarding cardiovascular effects in humans is that from a case report in which low blood pressure (80154 mm Hg) and tachycardia (160 beats per minute) were observed in a man shortly after he swallowed 30-40 mL of a varnish containing a nitrobenzene dye (Kumar et al. 1990). After gastric lavage, his blood pressure increased to 100/70 mm Hg and his heart rate decreased to 120 beats per minute. Blood pressure further increased to 106/74 and heart rate decreased to 99 beats per minute following an intravenous injection of methylene blue.

TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral

| Key [a] to figure | Species/ (Strain) | Exposure/ Duration/ Frequency (Specific Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|
| | **ACUTE EXPOSURE** | | | | | | |
| | **Death** | | | | | | |
| 1 | Rat (Carworth Farms) | once (GO) | | | | 91 M (LD50)<br>81 F (LD50) | Cody et al. 1981 |
| 2 | Rat (Sprague-Dawley) | once (GO) | | | | 48 M (death in 10/24) | Linder et al. 1990 |
| 3 | Mouse (B6C3F1/J) | once (GO) | | | | 40 M (10% mortality, but group size not given) | Evenson et al. 1989a |
| 4 | Rabbit (NS) | once (GW) | | | | 100 (death in 3 days, group size not given) | Parke 1961 |
| | ***Systemic*** | | | | | | |
| 5 | Rat (Alderley - Park) | once (GO) | Hemato | 10M | 15M (enlarged spleen, congestion, increased erythropoietic activity) | | Blackburn et al. 1988 |
| 6 | Rat (Sprague-Dawley) | once (GO) | Hemato | 8M | | 16 M (cyanosis) | Linder et al. 1990 |
| | | | Endocr | 48M | | | |
| | | | Bd Wt | 48M | | | |
| 7 | Rat (F-344) | once (GO) | Hemato | | | 25 M (28% methemoglobin; cyanosis) | Philbert et al. 1987b |

TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)

| Key [a] to figure | Species/ (Strain) | Exposure/ Duration/ Frequency (Specific Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|
| 8 | Rat (Alderley - Park) | once (G) | Hemato | 10M | | 20 M (cyanosis) | Reader et al. 1991 |
| | | | Bd Wt | 30M | | | |
| 9 | Mouse (B6C3F1/J) | once (GO) | Bd Wt | 48M | | | Evenson et al. 1989a |
| | **Neurological** | | | | | | |
| 10 | Rat (Sprague-Dawley) | once (GO) | | 32M | | 48 M (ataxia, loss of equilibrium) | Linder et al. 1990 |
| 11 | Rat (F-344) | once (GO) | | | | 20 M (splayed hind limbs and flaccid paralysis of fore limbs in germ-free rats) | Philbert et al. 1987b |
| | **Reproductive** | | | | | | |
| 12 | Rat (Alderley - Park) | once (GO) | | 10M | | 15 M (damaged germinal epithelium, degeneration and loss of spermatocytes and spermatids) | Blackburn et al. 1988 |
| 13 | Rat (Sprague-Dawley) | once (GO) | | 16M | | 32 M (increase in diploid and decrease in haploid cells; abnormal chromatin) | Evenson et al. 1989b |

**TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)**

| Key [a] to figure | Species/ (Strain) | Exposure/ Duration/ Frequency (Specific Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|
| 14 | Rat (Sprague-Dawley) | once (GO) | | | | 48 M (decreased sperm counts, decreased sperm motility, abnormal sperm morphology, degeneration and sloughing of both spermatocytes and spermatids) | Linder et al. 1988 |
| 15 | Rat (Sprague-Dawley) | once (GO) | | 8[b] M | | 16 M (damaged testicular epithelium, decreased spermatozoa, morphological changes in spermatozoa, histological changes in epididymis) | Linder et al. 1990 |
| 16 | Rat (Sprague-Dawley) | once (GO) | | 12M | | 30 M (decreased relative testes weight, altered testicular histopathology) | Moore et al. 1992 |
| 17 | Rat (Alderley-Park) | once (G) | | | 10M (increase in plasma LDH-C4 indicative of germ cell loss) | 30 M (Sertoli cell cytoplasmic retraction and vacuolation; spermatocytes loss) | Reader et al. 1991 |
| 18 | Mouse (B6C3F1/J) | once (GO) | | 32M | | 48 M (decreased testes weight, reduced numbers of N and 4N testicular cells, abnormalities in chromatin stucture from epididymal sperm.) | Evenson et al. 1989a |

**TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)**

| Key [a] to figure | Species/ (Strain) | Exposure/ Duration/ Frequency (Specific Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|
| | **INTERMEDIATE EXPOSURE** | | | | | | |
| | **Death** | | | | | | |
| 19 | Rat (Carworth Farms) | 8 wk ad libitum (W) | | | | 12.5 M (4/6 died ) 14.4 F (2/6 died) | Cody et al. 1981 |
| 20 | Rat (Sprague-Dawley) | 12 wk 5 d/wk (GO) | | | | 6 M (2/12 died) | Linder et al. 1986 |
| | **Systemic** | | | | | | |
| 21 | Rat (Carworth Farms) | 16 wk ad libitum (W) | Resp | 3.1 F | | | Cody et al. 1981 |
| | | | Cardio | 3.1 F | | | |
| | | | Gastro | 3.1 F | | | |
| | | | Hemato | 1.13M | 2.64M (moderate decrease in hemoglobin) | | |
| | | | Musc/skel | 3.1 F | | | |
| | | | Hepatic | 3.1 F | | | |
| | | | Renal | 3.1 F | | | |
| | | | Endocr | 3.1 F | | | |
| | | | Derm | 3.1 F | | | |
| | | | Ocular | 3.1 F | | | |
| | | | Bd Wt | 1.32 F | 3.1 F (significant, but unspecified decrease in bw gain after week 8) | | |

**TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)**

| Key [a] to figure | Species/ (Strain) | Exposure/ Duration/ Frequency (Specific Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|
| 22 | Rat (Carworth Farms) | 8 wk ad libitum (W) | Resp | 14.4 F | | | Cody et al. 1981 |
| | | | Cardio | 14.4 F | | | |
| | | | Gastro | 14.4 F | | | |
| | | | Hemato | | 4.7 M (mild decrease in hemoglobin)<br>6.0 F | | |
| | | | Musc/skel | 14.4 F | | | |
| | | | Hepatic | 14.4 F | | | |
| | | | Renal | 14.4 F | | | |
| | | | Endocr | 14.4 F | | | |
| | | | Derm | 14.4 F | | | |
| | | | Ocular | 14.4 F | | | |
| | | | Bd Wt | | | 4.7 M (bw gain reduced 43%)<br>6.0 F (bw gain reduced 23%) | |
| 23 | Rat (Sprague-Dawley) | 12 wk 5 d/wk (GO) | Hemato | | 0.75 [c] M (splenic hemosiderosis) | | Linder et al. 1986 |
| | | | Endocr | 6M | | | |
| | | | Bd Wt | 3M | 6M (16% bw loss during breeding period) | | |
| | **Immuno./Lymp** | | | | | | |
| 24 | Rat (Carworth Farms) | 16 wk ad libitum (W) | | 0.40 M<br>0.48 F | 1.13 M (increased spleen weight)<br>1.32 F | | Cody et al. 1981 |

**TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)**

| Key [a] to figure | Species/ (Strain) | Exposure/ Duration/ Frequency (Specific Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|
| 25 | Rat (Carworth Farms) | 8 wk ad libitum (W) | | | 4.7 M (increased spleen weight) 6.0 F | 12.5 M (spleen atrophy and 14.4 F fibrosis, hemosiderin deposition) | Cody et al. 1981 |
| 26 | Rat (Sprague-Dawley) | 12 wk 5 d/wk (GO) | | 0.75 M | 1.5 M (increased spleen weight) | | Linder et al. 1986 |
| | **Neurological** | | | | | | |
| 27 | Rat (Carworth Farms) | 90 day ad libitum (W) | | 1.13 M | | | Cody et al. 1981 |
| 28 | Rat (Carworth Farms) | 16 wk ad libitum (W) | | 3.1 F | | | Cody et al. 1981 |
| 29 | Rat (Carworth Farms) | 8 wk ad libitum (W) | | 14.4 F | | | Cody et al. 1981 |
| 30 | Rat (Sprague-Dawley) | 12wk 5 d/wk (GO) | | 3 M | | 6 M (ataxia, paresis equilibrium loss, muscle rigidity) | Linder et al. 1986 |
| | **Reproductive** | | | | | | |
| 31 | Rat (Carworth Farms) | 8 wk ad libitum (W) | | 14 F | | 4.7 M (testicular atrophy; decreased spermatogenesis; reduced testicular weight) | Cody et al. 1981 |

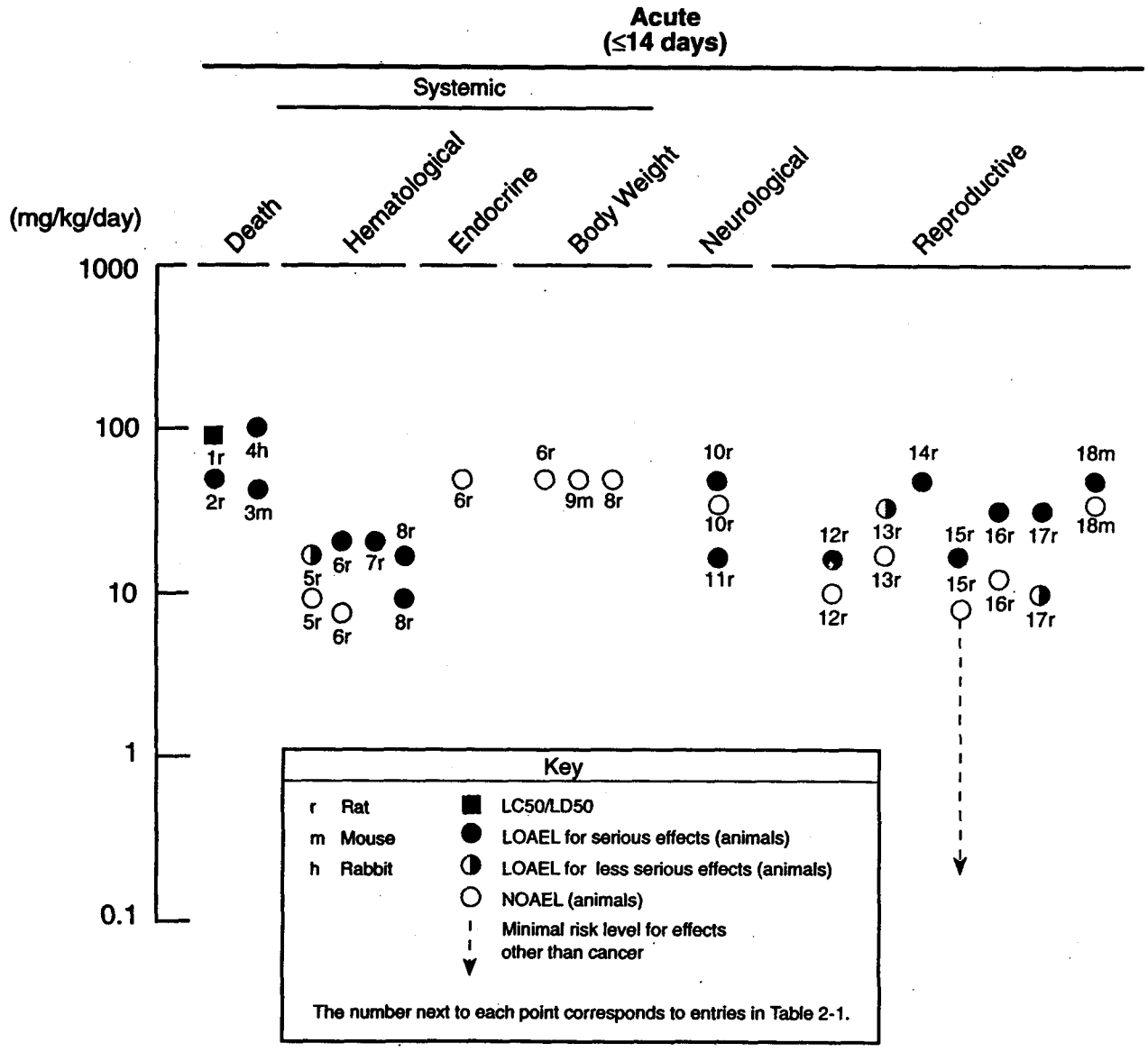
**TABLE 2-1 Levels of Significant Exposure to 1,3-Dinitrobenzene - Oral (continued)**

| Key [a] to figure | Species/ (Strain) | Exposure/ Duration/ Frequency (Specific Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|
| 32 | Rat (Carworth Farms) | 16 wk ad libitum (W) | | 1.13 M<br>3.1 F | 2.64 M (decreased testes weight, decrease in spermatogenesis) | | Cody et al. 1981 |
| 33 | Rat (Sprague-Dawley) | 12 wk 5 d/wk (GO) | | 0.75 M | 1.5 M (decreased testicular spem counts) | 3 M (no sperm in testis and epididymis cauda; decreased testis and epididymal weight; infertility) | Linder et al. 1986 |

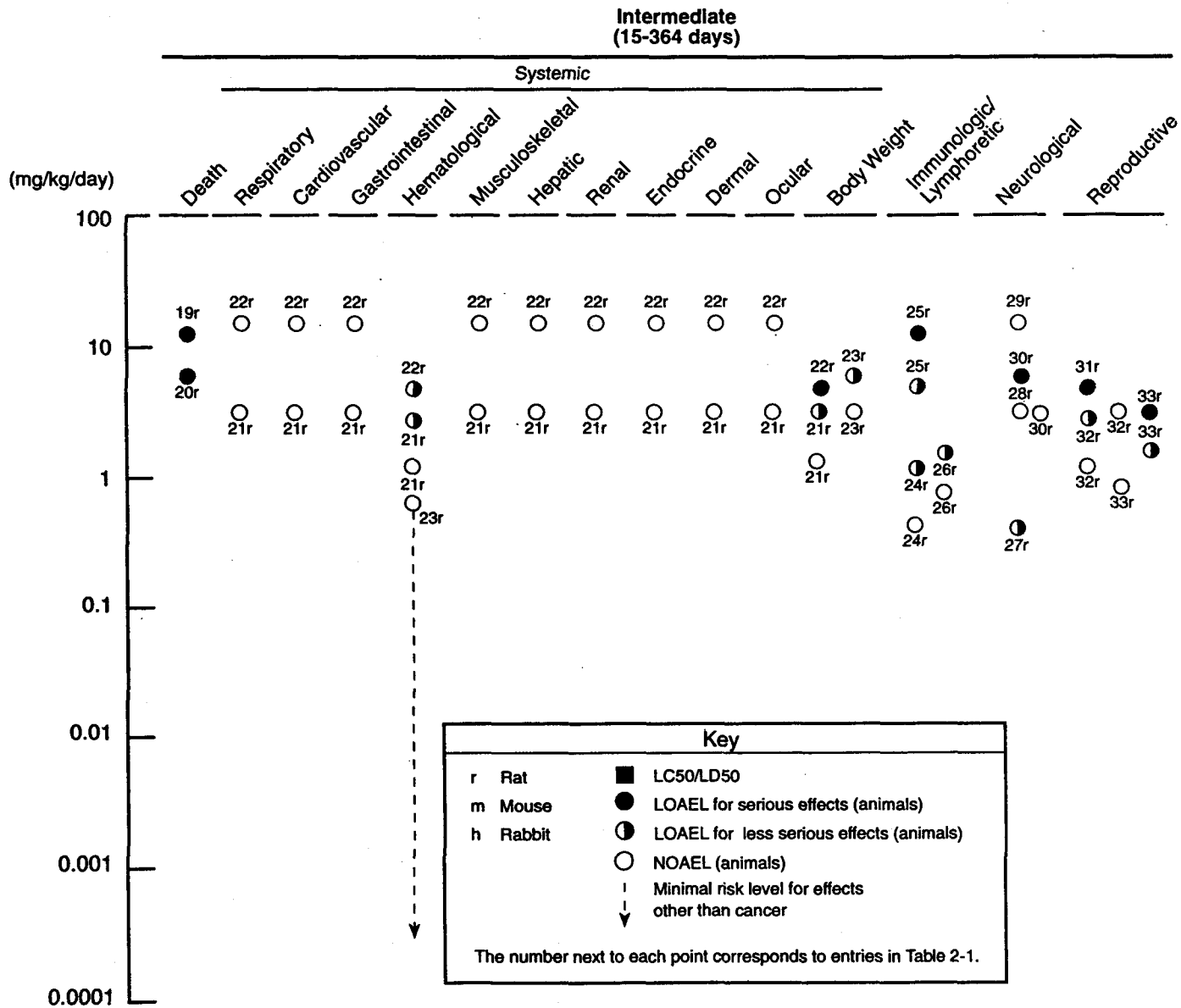
[a]The number corresponds to entries in Figure 2-1. Differences in levels of health effects between males and females are not indicated in Figure 2-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[b]Used to derive an acute oral Minimal Risk Level (MRL) of 0.008 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability)
[c]Used to derive an intermediate oral MRL of 0.0005 mg/kg/day; dose adjusted for intermittent exposure by multiplying by 5/7 and divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; bw = body weight (LOAEL column); Cardio = cardiovascular; CV = conventional; d = day(s); Derm = dermal; Endocr = endocrine; F = female; (G) = gavage; (GO) = gavage in oil; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LD50 = lethal dose, 50% kill; LDH-C4 = lactate dehydrogenase isozyme C4; LOAEL = lowest-observed-adverse-effect level; M = male; Musc/skel = musculoskeletal; N = haploid cells; 4N = polyploid cells; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; (W) = water; wk = week(s)

## Figure 2-1. Levels of Significant Exposure to 1,3-Dinitrobenzene



## Figure 2-1. Levels of Significant Exposure to 1,3-Dinitrobenzene – Oral (continued)



Information in animals is restricted to a single intermediate-duration study in rats in which no histopathologic alterations were observed in the heart and aorta of rats given up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water (Cody et al. 1981).

No studies were located regarding the cardiovascular effects in humans or animals after oral exposure to 1,3,5-TNB.

**Gastrointestinal Effects.** No studies were located regarding gastrointestinal effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

No histopathologic alterations were observed in the stomach, duodenu*m*-pylorus, ileum, and colon of rats administered up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water (Cody et al. 1981). No further data were located regarding 1,3-DNB and no information was available regarding 1,3,5-TNB.

**Hematological Effects.** No studies were located regarding hematological effects in humans after oral exposure to 1,3,5-TNB.

The primary effect of 1,3-DNB absorbed into blood is the formation of methemoglobin. For a detailed discussion on the mechanism of methemoglobinemia induction please see Section 2.3.5.

The only information regarding hematological effects of 1,3-DNB in humans is that of a man who swallowed 30-40 mL of nitrobenzene dye and was admitted to the hospital with peripheral and central cyanosis (Kumar et al. 1990). Evidence of hemolytic anemia was present. Methemoglobin was 37.2% after gastric lavage was performed, and was reduced to 5.7% after two injections of methylene blue.

No studies were located regarding hematological effects in animals after oral exposure to 1,3,5-TNB. In acute-duration studies in rats treated with 1,3-DNB, cyanosis was the first sign of acute toxicity and deficient blood oxygenation. The exposure doses in rats ranged from 16 to 180 mg/kg (Blackbum et al. 1988; Cody et al. 1981; Linder et al. 1988, 1990; Philbert et al. 1987b; Reader et al. 1991). In general, the effect was readily reversed when treatment with 1,ZDNB was discontinued. Mice treated once with up to 48 mg/kg 1,3-DNB did not appear to develop cyanosis, but the scope of this study

was primarily the assessment of reproductive end points and not hematological parameters (Evenson et al. 1989a).

When rats were exposed to low doses (0.4-3.1 mg/kg/day) of 1,3-DNB for 16 weeks, no overt signs of acute toxicity were seen (Cody et al. 1981). There was, however, a moderate decrease in hemoglobin levels after weeks 5 and 10, but they returned to control levels by week 14 (Cody et al. 1981). Splenic hemosiderosis was observed in all groups of rats treated with 0.75-6 mg 1,3-DNB by gavage for 12 weeks, including controls (Linder et al. 1986). Splenic hemosiderosis, which was minimal in controls and moderate to moderately severe at the highest dose level, is consistent with hemolytic anemia. No chronic-duration studies were located.

**Musculoskeletal Effects.** No studies were located regarding musculoskeletal effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

Information in animals is limited to a study in which no histopathologic alterations were observed in skeletal muscle (unspecified) of rats given up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water (Cody et al. 1981). No information was located regarding 1,3,5-TNB.

**Hepatic Effects.** No studies were located regarding hepatic effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

No histopathological alterations were seen in the liver of rats treated with up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water (Cody et al. 1981). No information was available regarding 1,3,5-TNB.

**Renal Effects.** No studies were located regarding renal effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

Only one study was located that examined the effect of 1,3-DNB on the kidneys (Cody et al. 1981). In that study, no histopathologic alterations were observed in the kidneys of rats administered up to

3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water. No information was located regarding 1,3,5-TNB.

**Endocrine Effects.** Levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (Prl), hypothalamic gonadotropin releasing hormone (GnRH), testosterone, and androgenbinding protein (ABP) were evaluated in male Sprague-Dawley rats from 3 hours to 2 weeks after treatment with a single oral dose of 32 mg/kg 1,3-DNB (Rehnberg et al. 1988).

Pituitary weights and weights of androgen-dependent accessory sex organs did not differ in treated animal as compared to the controls at any time point. Serum and pituitary levels of LH and Prl were not affected by I,3-DNB treatment at any time point examined. FSH concentration in serum was significantly higher 2 weeks after treatment, while pituitary FSH levels remained unchanged. These results led the investigators (Rehnberg et al. 1988) to suggest that 1,3-DNB exerts a direct effect on the testes and not through alterations in hypothalamic and pituitary control of gonadal function. No significant changes in pituitary weight were observed over a 14-day period in male rats exposed to a single oral dose of 8-48 mg/kg 1,3-DNB (Linder et al. 1990).

In intermediate-duration studies, no histopathologic alterations were observed in the pancreas, thyroids, adrenals, and pituitary of rats given up to 14 mg/kg/day 1,3-DNB for 8 weeks or 3 mg/kg/day 1,3-DNB for 16 weeks in the drinking water (Cody et al. 1981). Administration of up to 6 mg/kg/day 1,3-DNB by gavage for 12 weeks to rats did not result in alterations of the adrenal's weight (Linder et al. 1986). No further endocrine end point was assessed in the latter study.

**Dermal Effects.** No studies were located regarding dermal effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

Data in animals are provided by only one intermediate-duration study in rats (Cody et al. 1981). In that study, the investigators reported that no histopathological alterations were observed in the skin of rats given up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks in the drinking water. No data were available for 1,3,5-TNB.

**Ocular Effects.** No studies were located regarding ocular effects in humans after oral exposure to **1,3-DNB** or 1,3,5-TNB.

Very little information was available regarding ocular effects in animals. Exophthalmia was observed in rats given a single lethal dose of 1,3-DNB (Cody et al. 1981). This effect, according to the investigators, appeared to reflect a condition of general congestion prior to death. In an intermediateduration study (Cody et al. 1981), no histopathologic alterations were observed in the eyes of rats treated with up to 14 mg/kg/day 1,3-DNB for 8 weeks or 3 mg/kg/day for 16 weeks in the drinking water. No data were found regarding 1,3,5-TNB.

**Body Weight Effects.** No studies were located regarding body weight effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

In an acute-duration study, male Sprague-Dawley rats treated with a single dose of 848 mg/kg 1,3-DNB and examined over a 32-day period did not show significant decreases in body weight (Evenson et al, 1989b). In addition, the same group of investigators (Evenson et al. 1989a) noted no significant differences in body weight between treated and control groups of adult and pubertal (26 days old) male mice exposed to a single oral dose of 8-48 mg/kg 1,3-DNB. No significant changes in body weight were noted in male rats exposed to a single oral dose of 8-48 mg/kg 1,3-DNB and observed for 14 (Linder et al. 1990) or 175 days (Linder et al. 1988).

A significant reduction in body weight gain was noted in male and female rats exposed for 8. weeks to 4.7 and 6 mg/kg/day 1,3-DNB, respectively, via drinking water (Cody et al. 1981). Higher doses, 12.5 mg/kg/day in males and 14.4 mg/kg/day in females, induced frank weight loss (Cody et al. 1981). In another study by the same group of investigators (Cody et al. 1981), female rats exposed to 3 mg/kg/day in the drinking water for 16 weeks had a reduced rate of growth after 8 weeks and, at the end of 16 weeks, weight was significantly lower than for control females; growth rate of males was not affected by treatment with 1,3-DNB. Also, male rats treated by gavage with 6 mg/kg/day 1,3-DNB for 12 weeks experienced a significant decrease (16%) in body weight during a breeding period of a week (Linder et al. 1986). No information was located regarding 1,3,5-TNB.

### 2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after exposure to 1,3-DNB or 1,3,5-TNB.

Splenic enlargement and congestion, and increased erythropoietic activity were observed in the spleen from male rats (females were not tested) treated' with a single dose of 15 or 25 mg/kg 1,3-DNB and sacrificed at various intervals (2-96 hours) after dosing (Blackbum et al. 1988). This, however, is most likely an extramedullary response and is consistent with hemolytic anemia.

In intermediate-duration studies, spleen enlargement occurred in male and female rats treated with approximately 1 mg/kg/day 1,3-DNB in the drinking water for 16 weeks (Cody et al. 1981). Similar results were reported in rats administered 1.5 mg/kg/day 1,3-DNB by gavage for 12 weeks (Linder et al. 1986). Treatment of rats with doses of about 12-14 mg/kg/day 1,3-DNB in the drinking water for 8 weeks induced hemosiderin deposits in the spleen and spleen atrophy and fibrosis (Cody et al. 1981). No data were located for 1,3,5-TNB.

All reliable LOAEL values for immunological effects in each species and duration category for 1,3-DNB are recorded in Table 2-l and plotted in Figure 2-l.

### 2.2.2.4 Neurological Effects

The only information available regarding neurological effects in humans comes from a case report of an accidental poisoning of a man who swallowed varnish containing nitrobenzene dye and was admitted to the hospital in a deep coma. Based on the known properties of nitrobenzenes in general, the development of coma may have been secondary to methemoglobinemia (37.2%) and cyanosis.

No studies were located regarding neurological effects in animals after oral exposure to 1,3,5-TNB. Physical signs of neurotoxicity following acute-duration exposure to 1,3-DNB were manifested in slow movement, loss of movement, loss of equilibrium, and general hypoactivity in rats given single oral doses of 1,3-DNB ranging from 20 to 48 mg/kg (Linder et al. 1988, 1990; Philbert et al. 1987b).
Older adult rats seemed to be more susceptible to neurotoxicity following a single dose of 48 mg/kg of 1,3-DNB (Linder et al. 1990).

Studies of intermediate duration evaluating neurological effects are inconclusive. Administration of 6 mg/kg/day 1,3-DNB by gavage to male Sprague-Dawley rats (females not tested) for 12 weeks caused severe neurotoxic effects (impaired movement, paresis, loss of equilibrium, and muscle rigidity) in all animals (Linder et al. 1986). In another study, the activity level of male Cat-worth Farms rats (females not tested) given 0.4 and 1.1 mg/kg/day 1,3-DNB in drinking water for 16 weeks was measured with both activity wheels and activity platforms (Cody et al. 1981). In both treated groups, there was a significant increase in activity wheels relative to controls, but activity in platforms was not significantly greater than the level of activity of the control group. The same group of investigators (Cody et a!. 1981) found no histopathologic alterations in the brain and spinal cord from rats given up to 3 mg/kg/day 1,3-DNB for 16 weeks or 14 mg/kg/day for 8 weeks.

All reliable LOAEL values for neurological effects in each species and duration category for 1,3-DNB are recorded in Table 2-1 and plotted in Figure 2-1.

**2.2.2.5 Reproductive Effects**

No studies were located regarding reproductive effects in humans after oral exposure to 1,3-DNB or 1,3,5-TNB.

No studies were located regarding reproductive effects in animals after oral exposure to 1,3,5-TNB. Reproductive toxicity in the form of reduced testes and epididymis weight was consistently observed in rats exposed to a single oral dose (ranging from 25 to 50 mg/kg) of 1,3-DNB (Blackbum et al. 1988; Evenson et al. 1989a; Linder et al. 1988; Rehnberg et al. 1988). Reduced testicular weight was observed in two groups of adult Sprague-Dawley rats-younger and older animals-after a single 24 mg/kg dose of 1,3-DNB (Linder et al. 1990). However, older adult rats were more sensitive to 1,3-DNB-induced toxicity. Epididymal weight, testicular sperm head count, and cauda sperm reserves in older rats treated with 16 mg/kg of 1,3-DNB were all significantly lower than controls (Linder et al. 1990). In another study, male Sprague-Dawley rats (3-5/group) were given a single oral dose of 1,3-DNB in 1.5% dimethyl sulfoxide (DMSO) in corn oil at 0, 12, 30, or 60 mg/kg; animals were sacrificed 48 hours later (Moore et al. 1992). The lowest dose had no effect on the testis. At 30 mg/kg, degeneration and depletion of some of the late pachytene spermatocytes (phagocytosis and exfoliation) were observed. At 60 mg/kg, all of the pachytene spermatocytes and round spermatids were absent or degenerate. Relative testis weight was reduced at 30 and 60 mg/kg in a dose-related

manner. Based on the finding that urinary creatine was significantly increased at 60 mg/kg in the 24-hour period following dosing (a period consistent with Sertoli cell damage), the authors (Moore et al. 1992) concluded that a substantial proportion of testicular creatine is associated with the cells of the seminiferous epithelium and that creatinuria may serve as a marker for damage to these cells.

In contrast to these findings in .rats are observations made in prepubertal mice that testicular growth and development were not affected by 40 or 48 mg/kg of 1,3-DNB (Evenson et al. 1989a). In pubertal and adult mice, however, abnormal spermatogenesis and an increase in chromatin structure abnormality were apparent after a single dose of 48 mg/kg 1,3-DNB (Evenson et al. 1989a).

Testicular histopathology revealed that major early changes after exposure to 1,3-DNB consisted of degeneration of germinal epithelium and sloughing of both spermatocytes and spermatids which in turn resulted in reduced sperm counts and reduced sperm mobility (Blackbum et al. 1988; Evenson et al. 1989b; Linder et al. 1988, 1990; Reader et al. 1991). Disrupted spermatogenesis was also evidenced by a decrease in the number of seminiferous tubules in rats treated with 48 mg/kg of 1,3-DNB (Hess et al. 1988). At 5 weeks, these changes caused decreased fertilizing ability of spermatozoa and 91% of treated rats lost their fertilizing capability (Linder et al. 1990). However, these changes were partially reversible since at 5 months after exposure only 18% of rats had not recovered their reproductive capability (Linder et'al. 1990).

An indication that Sertoli cells may be targets in the seminiferous epithelium for early damage by 1,3-DNB came with the observatton of significantly increased levels of androgen-binding protein (ABP, released from Sertoli cells) in.seminiferous tubule fluid, interstitial fluid, and serum in rats treated with 15 and 32 mg/kg of 1,3-DNB, respectively (Reader et al. 1991; Rehnberg et al. 1988). Further examination of early toxic effects of 15 mg/kg of 1,3-DNB revealed vacuolization and cytoplasmic retraction in Sertoli cells within the first 24 hours after exposure (Blackbum et al. 1988). Similar observations of Sertoli cell damage were made when 1,3-DNB was administered at a dose of 30 mg/kg (Reader et al. 1991). Data from these studies support the notion that Sertoli cells may be first and primary targets of the toxic effects of I,3-DNB in seminiferous epithelium.

Plasma hormones and enzymes of testicular origin were used as markers for evaluation of acute testicular toxicity in rats treated with 1,3-DNB. Lactate dehydrogenase isozyme C4 (LDHC4) and ABP were both elevated after treatment with doses between 10 and 25 mg/kg of 1,3-DNB (Reader et

al. 1991). Testosterone levels were reduced after treatment with 10 and 32 mg/kg of 1,3-DNB (Reader et al. 1991; Rehnberg et al. 1988).

Adverse reproductive effects were also observed in rats exposed to 1,3-DNB in intermediate-duration studies. Significantly decreased spermatogenesis and atrophy of seminiferous tubules were observed after 12 weeks of treatment with 3 mg/kg/day 1,3-DNB by gavage (Linder et al. 1986). Testicular atrophy was also observed at 4.7 mg/kg/day after 8 weeks of treatment with 1,3-DNB in the drinking water (Cody et al. 1981). A slightly lower dose, 2.64 mg/kg/day, given for 16 weeks induced a decrease in testes weight and decreased spermatogenesis (Cody et al. 1981).

In female rats, administration of up to 3 mg/kg/day 1,3-DNB in drinking water for 16 weeks or up to 14 mg/kg/day for 8 weeks caused no significant alterations in the weight or histopathologic appearance of the ovaries (Cody et al. 1981).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in animals after acute- or intermediate-duration oral exposure are recorded in Table 2-l and plotted in Figure 2- 1.

#### 2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after oral exposure to 1,3-DNB or 1,3,5-TNB.

#### 2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after oral exposure to 1,3-DNB or 1,3,5-TNB.

Genotoxicity studies are discussed in Section 2.4.

#### 2.2.2.8 Cancer

No studies were located regarding cancer effects in humans or animals after oral exposure to 1,3-DNB or 1,3,5-TNB.

### 2.2.3 Dermal Exposure

Human studies presented in the section on dermal exposure are reports of accidental occupational exposure and one volunteer case study. Since precise levels of exposure in these studies are not known, the results in this section are not presented in a table.

#### 2.2.3.1 Death

No studies were located regarding death, in humans after dermal exposure to 1,3-DNB or 1,3,5-TNB.

In an early study, a single dermal application of an ointment containing 25% 1,3-DNB to 3 cats resulted in the death of a female cat 12 hours after dosing (White and Hay 1901). Limitations of this study include small sample size and lack of information on the amount applied. Information located in an abstract indicates that the derrnal $LD_{50}$ for 1,3-DNB in rabbits was 1,990 mg/kg, and that a dose of 2,000 mg/kg 1,3,5-TNB was not toxic when applied for 24 hours to the skin of rabbits, but no further details were provided (Desai et al. 1991).

#### 2.2.3.2 Systemic Effects

No studies were located regarding respiratory, gastrointestinal, or musculoskeletal, effects in humans or animals after dermal exposure to 1,3-DNB or 1,3,5-TNB.

**Cardiovascular Effects.** The only information available is the case of an investigator who selfapplied an amount of ointment containing 100 mg 1,3-DNB three times over a 24-hour period (White and Hay 1901). After the third application, he noticed that his heart rate had increased to about 100-120 beats per minute and did not return to pre-exposure rate (not specified) until 3 days later. No further information was provided.

**Hematological Effects.** No studies were located regarding hematological effects in humans after dermal exposure to 1,3,5-TNB. Limited information is available regarding hematological effects in humans after dermal exposure to 1,3-DNB. The case of an investigator who self-applied an amount of ointment containing 100 mg of 1,3-DNB 3 times over a 24-hour period is described in an early study (White and Hay 1901). After only two applications, he noticed that his lips, tongue, and fingernails

were blue. After the third application, cyanosis was evident; he recovered three days later. 1,3-DNB is easily absorbed through skin when in aqueous solution (Ishihara et al. 1976) and its main effect is induction of methemoglobin formation. A female worker who handled electronics parts immersed in a chemical mixture containing 0.5% weight-to-weight (w/w) 1,3-DNB became cyanotic and showed signs of anemia upon admission to the hospital 10 days later. The exposure conditions of the above case were duplicated with a volunteer worker (Ishihara et al. 1976). Methemoglobin levels reached a maximum of 11% four hours after exposure to 1,3-DNB. It was also confirmed that the main exposure route was dermal since 1,3-DNB readily permeated latex gloves used to handle immersed parts and 1,3-DNB was not detected in the volunteer's breathing-zone air. The fact that 1,3-DNB readily permeated the latex gloves used for protection has enormous implications in the occupational setting because it shows that this kind of protection is ineffective. Limitations of this study include small sample size, concomitant exposure to other chemicals, and lack of complete information *on* exposure dose.

No studies were located regarding hematological effects in animals after dermal exposure to 1,3,5-TNB. In one of the earliest reports on 1,3-DNB exposure, an ointment containing 25% (w/w) 1,3-DNB was applied to the backs of 3 cats (White and Hay 1901). All three developed classical symptoms of methemoglobinemia and cyanosis.

Methemoglobinemia also was observed in guinea pigs when a solution containing 0.5% (w/w) 1,3-DNB in a mixture of solvents characterized as water soluble was applied for 4 hours (Ishihara and Ikeda 1979). Methemoglobinemia did not develop when the solvent mixture contained less than 77.5% (w/w) ethylene glycol.

**Hepatic Effects.** No studies were located regarding hepatic effects in humans after dermal exposure to 1,3,5-TNB. In one case report of occupational exposure to 1,3-DNB (Ishihara et al. 1976), the exposed worker had palpable liver while her liver function tests were negative. This study is limited in that only a single case was described and functional tests were performed 10 days after the exposure.

No studies were located regarding hepatic effects in animals after dermal exposure to 1,3,5-TNB. In one of the earliest studies with 1,3-DNB, the investigators indicate that necropsy of a cat (1 out of 3)

to which an ointment containing 25% (w/w) 1,3-DNB was applied 8 days earlier showed fatty degeneration in the liver (White and Hay 1901). No further information was provided.

**Renal Effects.** No studies were located regarding renal effects in humans after dermal exposure to 1,3-DNB or 1,3,5-TNB.

Kidney inflammation was reported in a cat (1 out of 3) that received two applications of an ointment containing 25% (w/w) 1,3-DNB over a lo-day period (White and Hay 1901). According to the, investigators (White and Hay 1901), this was probably due to toxic nephritis. No further information was provided.

**Dermal Effects.** No studies were located regarding dermal effects of 1,3-DNB or 1,3,5-TNB in humans.

Data in animals are limited to a study in which no irritation was observed in the skin of guinea pigs after application of a formulation containing 0.5% 1,3-DNB (w/w) for 4 hours (Ishihara and Ikeda 1979). Data located in an abstract indicate that neither 1,3-DNB nor 1,3,5-TNB caused skin irritation when applied to the skin of rabbits, but no further details were provided (Desai et al. 1991).

**Ocular Effects.** No studies were located regarding ocular effects of 1,3-DNB or 1,3,5-TNB in humans.

Limited information was presented in an abstract indicating that 1,3-DNB caused mild eye irritation in rabbits, whereas 1,3,5-TNB caused severe irritation; no further details were provided (Desai et al. 1991).

### 2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after dermal exposure to 1,3-DNB or 1,3,5-TNB.

Limited data located in an abstract indicate that 1,3-DNB was not a skin sensitizer in guinea pigs, but 1,3,5-TNB caused a mild allergic reaction; no further details were provided (Desai et al. 1991).

#### 2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans after dermal exposure to 1,3,5-TNB. Very limited information is available regarding the neurological effects of 1,3-DNB. Headache (presumably of vascular origin) and general malaise were reported in a female w.orker who handled electronics parts immersed in a chemical mixture containing 0.5% (w/w) 1,3-DNB (Ishihara et al. 1976). Limitations of this study include small sample size, concomitant exposure to other chemicals, and lack of complete information on dose and duration of exposure.

No studies were located regarding neurological effects in animals after dermal exposure to 1,3-DNB or 1,3,5-TNB.

No studies were located regarding the following health effects in humans or animals after dermal exposure to 1,3-DNB or 1,3,5-TNB:

#### 2.2.3.5 Reproductive Effects

#### 2.2.3.6 Developmental Effects

#### 2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

#### 2.2.3.8 Cancer

No studies were located regarding cancer effects in humans or animals after dermal exposure,to 1,3-DNB or 1,3,5-TNB.

## 2.3 TOXICOKINETICS

Data regarding the toxicokinetics of 1,3-DNB or 1,3,5-TNB in humans are limited to information derived from two occupational studies and from a report in which the experimenter self-administered 1,3-DNB. These data provide qualitative evidence that 1,3-DNB may be absorbed in humans by the inhalation and dermal routes. There are no data regarding oral absorption of 1,3-DNB or 1,3,5-TNB

in humans. In animals, 1,3-DNB is rapidly absorbed by the oral route;. data from one study indicate that at least 70% of a single oral dose was absorbed. In animals, depending on the vehicle, 1,3-DNB can also be readily absorbed through the skin. It appears that polar vehicles facilitate absorption. No information was located regarding absorption of 1,3-DNB in animals by the inhalation route or of 1,3,5-TNB by any route of exposure. The mechanism by which 1,3-DNB and 1,3,5-TNB are transported to the tissues is not completely understood, but there is some evidence indicating that dinitrobenzenes can penetrate the red blood cell membrane.- No information was located regarding distribution patterns for 1,3-DNB or 1,3,5-TNB in humans for any route of exposure or in animals after inhalation or dermal exposure. The metabolism of 1,3-DNB in animals include both oxidative and reductive biotransformations, followed by conjugation. No information is available regarding metabolism in humans. Following oral exposure, the main route of excretion of 1,3-DNB metabolites .in animals is the urine. This also seems to be the case for humans after de'rmal exposure. No data were located regarding excretion of 1,3-DNB or metabolites after inhalation and oral exposure in humans or after inhalation and dermal exposure in animals. The toxicity of 1,3-DNB is related to its methemoglobin forming capacity in the red blood cells. A reactive metabolic intermediate has been .postulated as the responsible agent for the toxicity to the male reproductive organs, but the exact mechanism has not been elucidated.

### 2.3.1 Absorption

#### 2.3.1.1 Inhalation Exposure

Studies undertaken specifically to evaluate absorption of either 1,3-DNB or 1,3,5-TNB in humans after an inhalation exposure were not located. However, a study of an occupational exposure to 1,3-DNB showed that workers developed cyanosis within the first 24 hours after exposure (Okubo and Shigeta 1982). Inhalation was considered the major exposure pathway since skin contact was with 1,3-DNB in solid form. There was no information, however, on the amount of 1,3-DNB present in the air or on the amount of particulate 1,3-DNB deposited on the workers' skin.

Studies on the absorption of 1,3-DNB or 1,3,5-TNB in animals following inhalation exposure were not located.

### 2.3.1.2 Oral Exposure

There-are no quantitative data that describe the absorption of 1,3-DNB or 1,3,5-TNB following oral exposure in humans.

Limited information was located regarding quantitative absorption of 1,3-DNB in animals after oral exposure. Rabbits treated with randomly labeled $^{14}$C-1,3-DNB in arachis oil in singles doses of 50-100 mg/kg excreted 65-93% of the administered radioactivity in the urine within 2 days of dosing (Parke 1961). This indicated that at least that amount was absorbed from the gastrointestinal tract; Similar results were reported in rats in which excretion data suggested that at least 63% of a single oral dose was absorbed (Nystrom and Rickert 1987). Further evidence that 1,3-DNB is readily absorbed by the oral route is provided by the many studies which examined the toxicological effects of this compound administered orally (see Section 2.2.2). No information was located regarding 1,3,5-TNB.

### 2.3.1.3 Dermal Exposure

No studies were located regarding absorption of 1,3,5-TNB following dermal exposure in humans.

Data are very limited regarding absorption of 1,3-DNB following dermal exposure in humans. Evidence of dermal absorption was found in an early report in which an experimenter became cyanotic after self-applying an ointment containing 25% (w/w) 1,3-DNB (White and Hay 1901). Similar findings were described in a case of .a woman exposed to a solution containing 0.5% (w/w) 1,3-DNB at work and in a male volunteer (Ishihara et al. 1976).

No studies were located regarding absorption of 1,3,5-TNB following dermal exposure in animals.

The role that the solvent mixture plays in the absorption of 1,3-DNB was investigated in Hartley guinea pigs exposed to solutions of varying composition, but each containing 0.5% (w/w) 1,3-DNB (Ishihara and Ikeda 1979). The solvents were ethylene glycol and diethylene glycol at various concentrations, along with two different co-existing solutes, ammonium adipate and ammonium sebacate (Ishihara and Ikeda 1979). Animals were sacrificed immediately after a 4-hour dermal exposure. The animals that received 1,3-DNB in a solvent mixture containing 77.5% (w/w) or more

of ethylene glycol developed methemoglobinemia. Methemoglobinemia did not occur when the solvent mixture contained less than 77.5% ethylene glycol. Six dicarboxylic acids were also tested as co-existing solutes and only two, malonic and adipic acid in the presence of ,ethylene glycol and diethylene glycol, were able to induce methemoglobin formation. These two acids were also more water soluble than others and allowed water impregnation of stratum corneum by ethylene glycol. The authors suggested that the increased water content of stratum comeum in the presence of higher concentrations of ethylene glycol may enhance dermal absorption of 1,3-DNB (Ishihara and Ikeda 1979).

### 2.3.2 Distribution

#### 2.3.2.1 Inhalation Exposure

No studies were located regarding the distribution of 1,3-DNB or 1,3,5-TNB following inhalation exposure in humans or animals.

#### 2.3.2.2 Oral Exposure

No studies were located regarding distribution following oral exposure to 1,3-DNB or 1,3,5-TNB in humans.

Limited information was located regarding distribution of 1,3,5-TNB in animals. 1,3,5-TNB-DNA adducts were detected in the spleen of rats one day after being gavaged once with $^{14}$C-1,3,5-TNB (Reddy et al. 1991). DNA adducts were also found in the stomach and liver three days after dosing. Twenty-eight days after treatment, the residual adduct level in the liver and stomach was 25%, whereas in the spleen was still 100%. Tissue distribution of $^{14}$C after a single oral dose of 25 mg/kg of $^{14}$C-1,3-DNB was examined in Fischer 344 conventional (C) and ger*m*-free (GF) rats (Philbert et al. 1987b). The amount of $^{14}$C label in whole blood, plasma, pancreas, lungs, liver, kidney, adrenal, testis, quadriceps femoris muscle, sciatic nerve, white and brown fat, spinal cord, and brain stem was examined and was found to be higher in GF animals. The relative distribution of label in different organs was as follows: liver > white fat > brown fat > kidney > sciatic nerve > whole blood > plasma > testis > brain stem. The amount of label contained in the liver and brain of GF rats was 20 and 13 times greater, respectively, than in C rats (Philbert et al. 1987b). This finding illustrates the

importance of gastrointestinal tract microflora in the initial phases of 1,3-DNB biotransformation. The study is limited in that there is a lack of information on statistical significance of the data.

#### 2.3.2.3 Dermal Exposure

No studies were located regarding distribution of I,3-DNB or 1,3,.5-TNB foIlowing dermal exposure in humans or animals.

#### 2.3.2.4 Other Routes of Exposure

The administration of 1,3-DNB by the intraperitoneal route allows for almost complete absorption. The level of 1,3-DNB in blood was evaluated in rats and hamsters after a single intraperitoneal injection of 25 mg/kg of radioactive 1,3-DNB ($^{14}$C-1,3-DNB) (McEuen and Miller 1991). The peak blood concentration of $^{14}$C-1,3-DNB was 99.5 nmol/mL in rats and was reached within 1 hour of exposure. Rats had twice the blood level of $^{14}$C-1,3-DNB found in hamsters.

### 2.3.3 Metabolism

#### 2.3.3.1 Inhalation Exposure

No studies were located regarding metabolism following inhalation exposure to 1,3-DNB or 1,3,5-TNB in humans or animals.

#### 2.3.3.2 Oral Exposure

No studies were located regarding metabolism of 1,3-DNB or 1,3,5-TNB following oral exposure in humans.

Both oxidative and reductive biotransformations, followed by conjugation, have been demonstrated for the metabolism of 1,3-DNB in mammals. The metabolism of the three isomeric dinitrobenzenes administered as single oral doses to rats (25 mg/kg) has been determined and compared (Nystrom and Rickert 1987). Products formed through reduction of the nitro group predominated, and the major metabolites were 3-aminoacetanilide (22%), 4-acetamidophenylsulfate (6%), 1,3-diacetamidobenzene

(7%), and 3-nitroaniline-N-glucuronide (4%). These products and their proposed intermediates are diagramed in Figure 2-2.

The metabolism of an oral dose of 50-100 mg/kg $^{14}$C-1,3-DNB was followed in rabbits (Parke 1961). Of the metabolites detected in urine, 30% were conjugated with glucuronic acid and 6% with sulfate. The major urinary metabolites of 1,3-DNB were 2,4-diaminophenol (31%), 1,3-phenylenediamine (25%), 1,3-nitroaniline (18%), and 2-amino-4-nitrophenol (14%). Other minor metabolites comprising about 20% of the label were oxidation and reduction products and azoxy dimers.

Several *in vitro* metabolic studies on 1,3-DNB support the above in viva findings. 1,3-DNB appears not to be a substrate for rat hepatic or erythrocyte glutathione transferases (Cossum and Rickert 1985 1987) since no 1,3-DNB glutathione conjugates were identified. However, the chemical reaction of 3-nitrosonitrobenzene with glutathione has been shown to form the corresponding hydroxylamino and anilino metabolites (Ellis et al. 1992). Studies using rat testicular cells or a co-culture of testicular and Sertoli cells showed that 1,3-DNB is metabolized by nitro reduction to 1,3-nitroaniline through nitrosonitrobenzene and nitrophenyl hydroxylamine intermediates, without being conjugated to glutathione (Cave and Foster 1990; Foster 1989; Lloyd and Foster 1987).

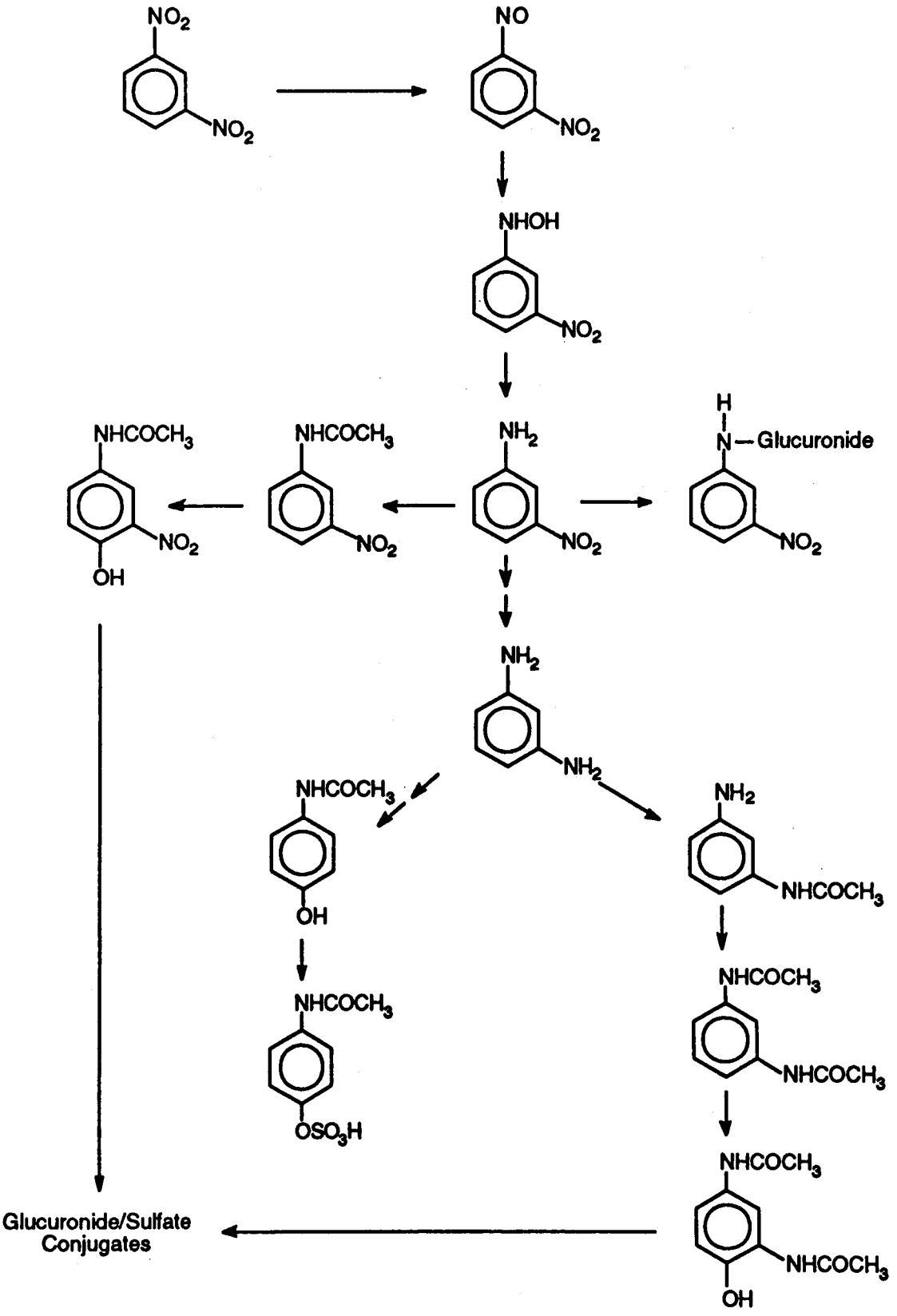
The relative rates of conversion of 1,3-DNB to nitroanilines were calculated in rat hepatocytes and microsomes from the slope of semilogarithmic plots of percentage 1,3-DNB remaining versus time. The half-life of 1,3-DNB was estimated to be 12 and 7 minutes in hepatocytes and microsomesi respectively, indicating a relatively rapid conversion rate (Cossum and Rickert 1985).

No studies were located regarding metabolism following oral exposure to 1,3,5-TNB in animals.

#### 2.3.3.3 Dermal Exposure

No studies were located regarding metabolism of 1,3,5-TNB following dermal exposure in humans. In the only study that measured 1,3-DNB metabolite production in humans after dermal exposure, the total production of both amino and nitro metabolites in urine was reported using 2,4-dinitrophenol as a standard (Ishihara et al. 1976). The results indicate that 1,3-DNB (in solution) rapidly penetrated skin and was also rapidly converted and excreted in urine. A maximum amount of amino and nitro metabolites was reached within the first hour after exposure and returned to normal levels after

**FIGURE 2-2. Proposed Pathway of Metabolism of 1,3-DNB in Rats and Hamsters***




* Adapted from Nystrom and Rickert 1987

10 hours. The limitations of this study are a small sample size (one person) and no detailed information on the nature of 1,3-DNB metabolites.

No studies were located regarding metabolism of 1,3-DNB or 1,3,5-TNB in animals after dermal exposure.

#### 2.3.3.4 Other Routes of Exposure

In mammals, the differences in metabolic processing of 1,3-DNB may play an important role in susceptibility to toxicity. The metabolism of 1,3-DNB was examined in rats and hamsters after a single intraperitoneal injection of 25 mg/kg of $^{14}$C-1,3-DNB (McEuen and Miller 1991). Hamsters were found to be much less sensitive than rats to the toxic effects of 1,3-DNB. Elimination of 1,3-DNB from blood was biphasic. The initial rapid phase was followed by a much slower one. Maximal blood levels of 1,3-DNB were 46 and 99 nmol/mL in hamsters and rats, respectively. In the urine, rats excreted more unconjugated metabolites and less phenolic metabolites than hamsters. The presence of unconjugated reductive metabolites in rats may in part be responsible for increased toxicity of 1,3-DNB. In another study, Sprague-Dawley rats and Syrian hamsters were exposed to increasing concentrations of 1,3-DNB. It was found that Syrian hamsters were more resistant to the toxic effects of 1,3-DNB (Obasaju et al. 1991). At the lowest dose (25 mg/kg), methemoglobin was 15% in hamsters compared to 83% in rats. The same was true for testicular damage observed within 48 hours in rats and absent in hamsters even when the dose was 50 mg/kg. This difference in susceptibility to toxic effects between the two species, rats and hamsters, is again probably due to differences in metabolism of 1,3-DNB.

### 2.3.4 Excretion

#### 2.3.4.1 Inhalation Exposure

No studies were located regarding excretion of 1,3-DNB or 1,3,5-TNB after inhalation exposure in humans or animals.

### 2.3.4.2 Oral Exposure

No studies were located regarding excretion following oral exposure to 1,3-DNB or 1,3,5-TNB in humans.

No studies were located regarding excretion following oral exposure to 1,3,5-TNB in animals. Following administration of a single oral dose of $^{14}$C-1,3-DNB to rabbits and rats, radioactivity accounting for more than 80% and 63% of the dose, respectively, was excreted in urine, indicating that the main route of excretion is via the urine (Nystrom and Rickert 1987; Parke 1961). Elimination of 1,3-DNB metabolites in urine was rapid and occurred within 48 hours. The major urinary metabolites in rabbits were 2,4-diaminophenol, 1,3-phenylenediamine, and 1,3-nitroaniline (Parke 1961).

### 2.3.4.3 Dermal Exposure

No studies were located regarding excretion of 1,3,5-TNB after dermal exposure in humans. In the only study that evaluated 1,3-DNB urinary metabolites in humans after dermal exposure, amino and nitro metabolites were reported as a single value using 2,4-dinitrophenol as a standard (Ishihara et al. 1976). Amino and nitro metabolites reached maximum levels within the 1st hour after exposure and returned to normal levels within 10 hours. The results indicate that 1,3-DNB was rapidly absorbed through skin and was also rapidly converted and excreted in urine. This study is limited by the small sample size (one person) and lack of information on the specific nature of 1,3-DNB metabolites.
No studies were located regarding excretion following dermal exposure to 1,3-DNB or 1,3,5-TNB in animals.

### 2.3.4.4 Other Routes of Exposure

Excretion of $^{14}$C-1 ,3-DNB was followed in urine and feces of Sprague-Dawley rats and Syrian hamsters after a single intraperitoneal dose of 25 mg/kg (McEuen and Miller 1991). More than 80% of the label was excreted in urine by both species within the first 24 hours. Rats needed less time to complete the 1,3-DNB elimination than did hamsters.

### 2.3.5 Mechanisms of Action

Two major systems have been identified as toxicity targets for 1,3-DNB: the red blood cell and the male reproductive system (see Section 2.2.2). In the red blood cell, 1,3-DNB induces formation of methemoglobin leading to cyanosis (Blackbum et al. 1988; Linder et al. 1988, 1990; Reader et al. 1991). In the male reproductive system, 1,3-DNB causes disruption of spermatogenesis resulting in hypospermia, poor sperm quality, and infertility (Blackburn et al. 1988; Hess et al. 1988; Linder et al. 1988). Whether adverse hematological and reproductive effects are caused by the same mechanism of action remains unresolved.

Reduction of the nitrogroup(s) of 1,3-DNB is a reaction that predominates over oxidative pathways in mammals. Reduction of the nitro groups produces reactive nitroaromatic radical anions which redox cycle to produce other reactive, toxic species such as superoxide anion (Mason and Holzman 1975; Wardman and Clarke 1976). Redox cycling of these intermediates probably causes the methemoglobinemia associated with exposure to 1,3-DNB (Kiese 1974). Methemoglobinemia is defined as a methemoglobin concentration of greater than 1%, and it results from iron in the normal ferrous state being oxidized to the ferric state at a rate that exceeds the. erythrocyte's reducing capacity. Methemoglobin is unable to combine reversibly with oxygen and carbon dioxide and also causes a shift in the oxygen dissociation curve toward increased oxygen affinity, preventing the transfer of oxygen from the blood to the tissues.

Within the reproductive system the prime target for 1,3-DNB toxicity appears to be the Sertoli cell. Results from numerous studies support this hypothesis (Blackbum et al. 1988; Hess et al. 1988; Linder et al. 1988). As previously mentioned, some investigators have suggested that testicular damage may be related to tissue hypoxia, which results from impaired oxygen transport as a consequence. of methemoglobinemia (Linder et al. 1988). It appears, however, that reduction of 1,3-DNB to reactive species such as nitrophenylhydroxylamine and nitrosonitrobenzene. are involved in the testicular toxicity of 1,3-DNB (Cave and Foster 1990; Ellis and Foster 1992). In studies using rat testicular cells or a co-culture of testicular and Sertoli cells, 1,3-DNB was metabolized by nitro reduction to 1,3-nitroaniline through nitrosonitrobenzene and nitrophenyl hydroxylamine intermediates, without being conjugated to glutathione (Cave and Foster 1990; Foster 1989; Lloyd and Foster 1987). Of all the intermediates tested, only nitrosonitrobenzene was able to induce histological changes similar to

those seen with 1,3-DNB when reintroduced into cell cultures (Foster 1989). The specific mechanism by which the reactive intermediate might induce cell damage is unknown.

## 2.4 RELEVANCE TO PUBLIC HEALTH

The general population is not likely to be exposed to either 1,3-DNB or 1,3,5-TNB. Exposure to both compounds is possible around Army ammunition plants. Occupational or accidental exposure to 1,3-DNB and 1,3,5-TNB may also occur in industries using these two compounds in manufacturing processes (e.g., explosives, plastics, dyes).

The major effects observed in animals after exposure to 1,3-DNB are methemoglobin formation and testicular damage at doses that are higher than 1 mg/kg. Both effects are common to other nitroaromatic compounds. The biochemical changes that occur in the blood, primarily methemoglobin formation, lead to oxygen deprivation in the tissues, and then to cyanosis and neurotoxicity.

For the general population, oral exposure to 1,3-DNB and 1,3,5-TNB is the most likely exposure route. It can occur through ingestion of contaminated water; however, the solubility of these compounds in water is quite low (500 ppm and 3,500 ppm, respectively). Inhalation exposure and dermal exposure to 1,3-DNB and 1,3,5-TNB present in air are less likely because of their low volatility.

No deaths have been reported in humans from exposure to either 1,3-DNB or 1,3,5-TNB. Information on the effects that occur in humans in response to 1,3-DNB exposure comes from case reports of accidental poisoning, from studies of occupationally exposed workers and from a study in which a subject self-administered 1,3-DNB for research purposes.

No information has been located regarding human exposure to 1,3,5-TNB. Acute exposure of humans to 1,3-DNB causes symptoms that are the result of increased levels of methemoglobin in the blood which in turn causes oxygen deprivation in the tissues. Among the first of these symptoms is cyanosis. Other signs of exposure of humans in occupational settings have been associated with mild central nervous system intoxication manifested by headaches and general malaise. No studies were located regarding chronic exposure to 1,3-DNB in humans.

Studies in animals support the observations of the toxic effects in humans. Moreover, results from animal studies indicate that other toxic effects could be associated with exposure to 1,3-DNB. These include testicular damage, decreased reproductive function, splenomegaly, and/or spleen atrophy.

**Minimal Risk Levels for 1,3-DNB and 1,3,5-TNB**.

***Inhalation MRLs.***

No inhalation MRLs were derived for 1,3-DNB or 1,3,5-TNB due to lack of human and animal data.

***Oral MRLs.***

An MRL of 0.08 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to 1,3-DNB.

The acute oral MRL was based on a NOAEL for testicular toxicity in male rats administered a single dose of ≥16 mg 1,3-DNB/kg in corn oil and sacrificed 14 days later (Linder et al. 1990). No adverse effects were observed at 8 mg 1,3-DNB/kg. Effects observed at ≥16 mg/kg included substantial damage to the testicular germinal epithelium, reduction in epididymal weigh, and decreased number and morphological changes in spermatozoa. Histological changes, including luminal debris and atypical cells and hypospermia were noted at ≥16 mg/kg. Cyanosis was seen at dose levels ≥16 mgJkg, and neurotoxicity and increased mortality occurred at 48 mg/kg. These results are supported by a number of studies in animals that have identified the male reproductive system as a target for 1,3-DNB toxicity (Blackburn et al. 1988; Moore et al. 1992; Reader et al. 1991).

An MRL of 0.0005 mg/kg/day has been derived for intermediate oral exposure (15-364 days) to 1,3-DNB.

The intermediate oral MRL was based on a LOAEL for splenic hemosiderosis in male rats administered 0.75 mg/kg/day 1,3-DNB by gavage in acetone/corn oil solution 5 days/week for 12 weeks (Linder et al. 1986). This dose-related response was minimal in controls and moderate to moderately severe at the highest dose level tested, 6 mg/kg/day. Splenic enlargement was also reported at 1.5 mg/kg/day. Adverse testicular effects were observed with doses of 1,3-DNB

≥1.5 mg/kg/day. Altered spermatogenesis was noted at ≥3 mg 1,3-DNB/kg/day. The observed splenic effects are considered secondary to the hematoxicity of 1,3-DNB and are supported by increased erythropoietic activity in rats in a study by Blackburn et al. (1988) and hemosiderosis in rats in a study by Cody et al. (1981), and are consistent with hemolytic anemia. The hematological effects of 1,3-DNB are consistent with effects produced by other nitroaromatic compounds, reinforcing the toxicological significance.

No MRL has been derived for chronic oral exposure to 1,3-DNB, or for acute-, intermediate-, or chronic-duration oral exposure to 1,3,5-TNB due to lack of data.

**Death.** No deaths have been reported in humans from exposure to either 1,3-DNB or 1,3,5-TNB. Death has been observed in rats, rabbits, and mice after oral exposure to 1,3-DNB (Cody et al. 1981; Evenson et al. 1989a; Linder et al. 1990; Parke 1961). Death has also been reported in rats after oral exposure to 1,3,5-TNB (Desai et al. 1991). However, it is unlikely that amounts of 1,3-DNB or 1,3,5-TNB sufficient to cause death could be ingested by humans from environmental exposures such as living close to ammunition plants or those employed in the dyestuffs, plastics, rubber, and other industries.

**Systemic Effects.**

***Respiratory Effects.*** Slight dyspnea upon exertion was reported in some of the workers after ah inhalation exposure to 1,3-DNB (Okubo and Shigeta 1982). Six factory workers were cleaning crystallized 1,3-DNB from a tank and had only gauze masks and rubber gloves for protection. No exposure data was available in that study. Shallow breathing was reported in a subject that ingested a varnish containing a nitrobenzene dye (Kumar et al. 1990), but this may have been secondary to the fact that the subject was in a coma and cyanosis had developed. No studies in animals were found on respiratory effects after exposure to 1,3-DNB other than a report in which no histopathologic alterations were seen in the lungs of rats exposed orally for 8-16 weeks (Cody et al. 1981). Data were not located for 1,3,5-TNB. The information available; although scant, do not seem to indicate that the respiratory system is a target for 1,3-DNB or 1,3,5-TNB.

***Cardiovascular Effects.*** Palpitations, low blood pressure, and tachycardia were described in subjects exposed to 1,3-DNB by the inhalation (Oknbo and Shigeta 1982), oral (Kumar et al. 1990), and

dermal (White and Hay 1901) routes of exposure. These responses are consistent with effects of organic nitrates. 1,3-DNB is an organic nitrate and shares many of the cardiovascular properties of therapeutic nitrates. Organic nitrates induce relaxation of the vascular smooth muscle which can result in peripheral vasodilation and a fall in blood pressure followed by a compensatory vasoconstriction (Abrams 1980). The general information available on organic nitrates suggests that exposure to 1,3-DNB or 1,3,5-TNB at ammunition waste sites or at work places where these chemicals are used may lead to adverse cardiovascular effects.

***Hematdogicd Effects.*** Induction of methemoglobin formation is one of the first hematological effects to occur after exposure to nitrobenzene compounds, including 1,3-DNB, by any route of administration (Ishihara et al. 1976; Kumar et al. 1990; Okubo and Shigeta 1982). As a result of oxygen deprivation and increased methemoglobin levels, cyanosis becomes apparent within the first 24 hours after exposure (White and Hay 1901). The mechanism of methemoglobin formation is discussed in section 2.3.5. After exposure to 1,3-DNB, mild-to-moderate anemia may occur and its severity depends on the duration of the exposure (Ishihara et al. 1976; Kumar et al. 1990; Okubo and Shigeta 1982). This small number of studies indicate that 1,3-DNB causes hematological effects in humans shortly after exposure. Although there are no data on high-level or intermediate exposure, it is reasonable to expect that higher levels of 1,3-DNB and longer exposure times would cause severe toxic effects. Studies in animals support findings of these toxic effects in humans and suggest that metabolic processing of 1,3-DNB plays an important role in susceptibility. to hematological effects. For example, an intraperitoneal dose of 25 mg/kg 1,3-DNB caused 15% methemoglobin in hamsters compared with 80% in rats (Obasaju et al. 1991). Another study found that. after administration of the same. dose of 1,3-DNB to hamsters and rats, blood levels of 1,3-DNB in hamsters were half those found in rats (McEuen and Miller 1991). Other differences in the metabolic disposition of 1,3-DNB between hamsters and rats were that rats had higher blood levels of the metabolite nitroaniline and excreted more unconjugated and less phenolic metabolites in the urine (McEuen and Miller 1991). All together, these data suggest that metabolic activation may be needed for hematotoxicity and that this metabolic activation is species-specific. No information was found regarding hematological effects in humans or animals after exposure to 1,3,5-TNB. An intermediate oral MRL was derived based on hematological effects in male rats administered 0.75 mg/kg/day 1,3-DNB by gavage for 12 weeks (Linder et al. 1986).

**Hepatic Effects**. Results of the studies on hepatic effects in humans after exposure to 1,3-DNB are inconclusive. In one case report of occupational exposure to 1,3-DNB, the exposed worker had palpable liver and jaundice while her liver function tests were negative (Ishihara et al. 1976). This study is limited in that it describes only one case and there is no information on the dose. In addition, functional tests were performed 10 days after the exposure occurred. In another study, hepatic transaminase levels (SGOT and SGPT) were within normal limits after a single acute-duration exposure to 1,3-DNB (Okubo and Shigeta 1982). Bilirubin was also found in all urine samples from exposed workers who were strongly positive for urobilinogen, indicating an unspecified degree of hepatobiliary disease. All exposed workers were followed for 10 years after exposure and showed no long-term adverse effects. This study is limited in that there are no data on the dose of 1,3-DNB and the functional tests were performed 9 days after exposure. Studies in animals provided little information. Exposure of rats to 1,3-DNB for 8 or 16 weeks in the drinking did not result in histopathologic alterations in the liver (Cody et al. 1981). Based on the available information and the lack of chronic-duration exposure data in humans or animals, it is difficult to estimate whether exposure to 1,3-DNB at hazardous waste sites or in industrial settings will lead to adverse hepatic effects. No information was found regarding hepatic effects in humans or animals after exposure to 1,3,5-TNB.

***Renal Effects***. Although no adverse effects on renal function have been reported, elevated levels of urobilinogen were found in workers after inhalation exposure to an unspecified amount of 1,3-DNB (Okubo and Shigeta 1982). It took approximately 50 days for urobilinogen to return to normal .levels. The only information located regarding renal toxicity in animals after exposure to 1,3-DNB was from an early study in which kidney inflammation was observed in a cat after dermal application of 1,3-DNB (White and Hay 1901); the dose applied was lethal. Persons exposed to high levels of 1,3-DNB by any of the three routes may have an increased risk of renal toxicity. It is not clear whether chronic exposure to very low levels of 1,3-DNB by any of the three routes might cause renal toxicity.

No studies were located regarding renal toxicity in humans or animals after exposure to 1,3,5-TNB. Therefore, it is not known if adverse renal effects would occur following inhalation, oral, or dermal exposure to 1,3,5-TNB.

***Body Weight Effects.*** No information was located regarding body weight effects in humans after inhalation, oral, or dermal exposure to 1,3-DNB or 1,3,5-TNB. One single oral intermediate-duration study showed that 1,3-DNB administered in drinking water for 8 weeks can lead to reduced growth rate (4-6 mg/kg/day) or even to body weight loss (12-14 mg/kg/day) (Cody et al. 1981). These responses could not be explained solely by reduced food consumption, but reduced water intake and dehydration may have played a role. The relevance of these findings to effects in humans is difficult to ascertain.

**Immunological and Lymphoreticular Effects.** No studies were located regarding immunological effects in humans after exposure to 1,3-DNB or 1,3,5-TNB. Studies in animals have not assessed the immune response after exposure to 1,3-DNB or 1,3,5-TNB, but spleen enlargement was reported in rats in acute- (Blackbum et al. 1988) and intermediate-duration (Cody et al. 1981; Linder et al, 1986) oral studies. Spleen enlargement, however, was probably a secondary response to the methemoglobinemia resulting from 1,3-DNB intake. Results from tests in guinea pigs showed that 1,3-DNB was not a skin sensitizer and that 1,3,5-TNB was mildly allergenic (Desai et al. 1991). Based on the information available, it is not known whether adverse immunological effects would occur in humans following inhalation, oral, or dermal exposure to 1,3-DNB or 1,3,5-TNB.

**Neurological Effects.** Very limited information is available regarding the neurological effects of 1,3-DNB. Slight headache, nausea, dizziness, and fatigue were symptoms reported in workers after inhalation exposure to 1,3-DNB (Okubo and Shigeta 1982). Headache accompanied a single dermal exposure to 1,3-DNB (Ishihara et al. 1976). These symptoms are probably the result of oxygen deprivation due to the presence of increased methemoglobin in the blood or to vasodilation of cerebral blood vessels. Acute-duration studies in animals have reported ataxia (weakness, loss of balance, flaccid paralysis) at doses that induced cyanosis (Cody et al. 1981; Linder et al. 1988, 1990; Philbert et al. 1987b). There was also ,a difference in susceptibility to the neurotoxic effects of 1,3-DNB between older and younger rats, older adult animals being more sensitive (attributed to reduced metabolism of 1,3-DNB) than younger ones (Linder et al. 1990). Results from intermediate-duration oral studies provided conflicting data. Ataxia was reported in rats given 6 mg/kg/day 1,3-DNB by gavage for 12 weeks (Linder et al. 1986), but neither ataxia nor histopathological alterations in the brain or spinal cord were observed in rats treated with 12-14 mg/kg/day 1,3-DNB in drinking water for 8 weeks (Cody et al. 1981). The different manner of administering the compound between these two studies may have contributed to the different responses. Also, increased activity in a platform was

seen in rats treated with a relatively low dose (0.4 mg/kg/day) of 1,3-DNB for 90 days in drinking water (Cody et al. 1981). This indicates that low 1,3-DNB doses may induce subtle neurological effects, which were not assessed in other studies. The existing information would suggest that adverse neurological effects appear at hematotoxic exposure levels. The information available is insufficient to determine whether long-term exposure to low levels of 1,3-DNB might affect the nervous system.

No studies were located regarding neurological effects after exposure to 1,3,5-TNB in humans or animals. Therefore, it is not known if inhalation, oral, or dermal exposure to 1,3,5-TNB would cause adverse neurological effects.

**Reproductive Effects.** Studies in humans have not addressed whether adverse reproductive effects occur after exposure to either 1,3-DNB or 1,3,5-TNB. However, adverse reproductive effects were observed in male rats, mice, and ‘hamsters (females were not tested) after a single or repeated oral administration of 1,3-DNB. The Sertoli cell has been suggested as the prime target for 1,3-DNB toxicity (Blackbum et al. 1988; Hess et al. 1988; Reader et al. 1991). Because the Sertoli cells have been shown to be involved in the control of spermatogenesis, damage to them could precipitate the wide range of effects seen in germ cells (Blackbum et al. 1988). Reduced testes and epididymis weights, disruption of spermatogenesis, hypospermia, poor sperm quality, and infertility were consistent findings regarding reproductive toxicity (Blackbum et al. 1988; Cody et al. 1981; Evenson et al. 1989a; Linder et al. 1986, 1988; Moore et al. 1992; Reader et al. 1991; Rehnberg et al. 1988). Susceptibility to reproductive toxicity of 1,3-DNB appears to be different in older and younger animals (Linder et al. 1990); the authors suggested that reduced metabolism in older animals led to greater bioavailability, implying that the parent compound may be the toxic entity. The specific mechanism of 1,3-DNB toxicity has not been elucidated. Some have suggested that testicular damage may be related to tissue hypoxia (Linder et al. 1988), which is the result of increased methemoglobin formation.

Different susceptibility among species to reproductive effects seems also related to the metabolism of 1,3-DNB. Rats were much more susceptible to adverse reproductive effects of 1,3-DNB than hamsters (McEuen and Miller 1991; Obasaju et al. 1991). This was correlated with the fact that blood levels of 1,3-DNB in the hamster reached only half those found in the rat and that blood levels of the metabolite 1,3-nitroaniline were higher in the rat (McEuen and Miller 1991). Furthermore, rats excreted more unconjugated and less phenolic metabolites than hamsters. Results from studies with rat

Sertoli/germ cell cocultures suggest that reactive metabolic intermediates such as nitrosonitrobenzene and nitrophenylhydroxylamine may be responsible for the testicular toxicity of 1,3-DNB (Cave and Foster 1990). An acute oral MRL was derived based on reproductive effects in male rats treated with a single dose of 16 mg/kg 1,3-DNB by gavage (Linder et al. 1990).

Based on the findings reported in these studies, the possibility of adverse effects occurring in human males following exposure to sufficiently high levels of 1,3-DNB cannot be excluded. As stated earlier, these higher 1,3-DNB levels are not likely to be present in the vicinity of ammunition plants.

**Developmental Effects.** Studies in humans or animals have not investigated whether adverse developmental effects occur as a result of exposure to 1,3-DNB or 1,3,5-TNB. Therefore, it is not known if inhalation, oral, or dermal exposure to 1,3-DNB or 1,3,5-TNB would cause adverse developmental effects.

**Genotoxic Effects.** There were no studies available regarding the genotoxicity of 1,3-DNB or 1,3,5-TNB in either humans or animals *in vivo.* One study was located that tested the effects of 1,3-DNB on rat liver cells. No significant increase in deoxyribonucleic acid (DNA) damage was observed (Probst et al. 1981). The remaining studies were eithef bacterial or fungal assays for mutagenicity, DNA damage, or mitotic recombination. The results for the *Salmonella typhimurium* mutagenicity tests were dependent on the strain of bacteria and test used. Positive responses were observed in strains TA98, TA100, TA1538, TA1537, TA1535, and D3052. Since strains TA98, TA1538, and TA1537 are sensitive to frameshift mutations and strains TA100 and TA1535 are sensitive to base-pair substitutions, positive responses in each of these strains suggest that 1,3-DNB produces both types of gene mutations in S. typhimurium (Chiu et al. 1978; Furukawa et al. 1985; Gamer and Nutman 1977; Kaden et al. 1979; Kerklaari et al. 1987; McGregor et al. 1980; Melnikow et al. 1981; Probst et al. 1981; Shimizu et al. 1983; Spanggord et al. 1982b). Two groups of investigators compared mutagenicity results using normal strains and strains deficient in nitroreductase (TA100NR or TA100NR3). The results were positive for the normal strains (TA100, TA98, and TA1538) but negative for the nitroreductase-deficient strains (Kerklaan et al. 1987; Spanggord et al. 1982b). This supports a well-documented notion that the mutagenicity observed in normal strains is due to endogenous bacterial reduction of the nitro groups (Chiu et al. 1978; Kerklaan et al. 1987; Probst et al. 1981; Shimizu et al. 1983; Spanggord et al. 1982b). Human intestinal flora contains nitroreducing bacteria, and it is therefore realistic to consider that ingestion of nitrobenzene compounds

may lead to mutagenic effects in humans. *Escherichia coli* was also examined for gene mutations following treatment with 1,3-DNB. Two strains were used: WP2 and WP2 uvrA. The results were negative for both, strains (Probst et al. 1981). Another study tested E**.** coli for DNA damage following 1,3-DNB treatment. According to the paper, concentrations of 1-10 mg/plate produced bacterial toxicity, but it was not clear whether these exposures produced DNA damage or any other form of genotoxicity (McGregor et al. 1980). A fungal study tested the effects of 1,3-DNB on mitotic recombination in *Succharomyces cerevisiae***.** Doses of up to 32 mg/mL were administered, but no genotoxic effects of any kind were observed either with or without metabolic activation (McGregor et al. 1980). Refer to Table 2-2 for a further summary of the genotoxic effects of 1,3-DNB exposure.

A few studies were located that tested the effects of 1,3,5-TNB on mutagenicity, DNA damage, or mitotic recombination, As with 1,3-DNB, 1,3,5-TNB was not significantly mutagenic in the Sulrnonellu strain deficient in nitroreductase, but gene mutations were observed in strains containing the enzyme (Spanggord et al. 1982b). *E. coli* was used to test the DNA-damaging capabilities of 1,3,5-TNB. All concentrations produced bacterial toxicity, but it was unclear whether DNA damage occurred (McGregor et al. 1980). 1,3,5-TNB did not affect mitotic recombination or produce any other observable genotoxic effect in S. *cerevisiue* (McGregor et al. 1980). Refer to Table 2-3 for a further summary of the genotoxic effects of 1,3,5-TNB exposure.

Unfortunately, the lack of human and animal exposure data makes it difficult to determine whether or not 1,3-DNB and 1,3,5-TNB are genotoxic. The available *in vitro* studies indicate that both chemicals have mutagenic potential in S. *typhimurium* bacteria. *S. typhimurium* is a classic system used to evaluate chemicals for their capacity to induce heritable mutations that potentially can occur in humans (Prival 1983). The observed mutagenicity, however, seems to arise from a derivative that is produced from nitroreduction. This nitroreduction occurs in rabbits (Parke 1961), rats (McEuen and Miller 1991; Nystrom and Rickert 1987), hamsters (McEuen and Miller 1991), cultured rat Sertoli cells (Cave and Foster 1990; Cossum and Rickert 1987; Lloyd and Foster 1987), and rat hepatocytes (Cossum and Rickert 1985). Since it is likely that the same nitroreduction process occurs in humans, then 1,3-DNB and 1,3,5-TNB may be considered potential human genotoxins.

**Cancer.** There is no information regarding carcinogenicity of 1,3,5-TNB or dinitrobenzenes including 1,3-DNB. Because of the lack of data regarding the carcinogenicity of 1,3-DNB and 1,3,5-TNB, EPA has placed them in Group D, not classifiable as to carcinogenic potential (IRIS 1994).

## TABLE 2-2. Genotoxicity of 1,3-DNB *In Vitro*

| Species (test system) | End point | Results: With activation | Results: Without activation | Reference |
|---|---|---|---|---|
| Prokaryotic organisms: | | | | |
| *Salmonella typhimurium* (TA100, TA100/GSH-) | Gene mutation | No data | + | Kerklaan et al. 1987 |
| *S. typhimurium* (TA100NR) | Gene mutation | No data | – | Kerklaan et al. 1987 |
| *S. typhimurium* (TA677) | Gene mutation | (+) | No data | Kaden et al. 1979 |
| *S. typhimurium* (TA1538) | Gene mutation | +[a] | + | Garner and Nutman 1977 |
| *S. typhimurium* (TA98) | Gene mutation | No data | + | Chiu et al. 1978 |
| *S. typhimurium* (TA100) | Gene mutation | No data | – | Chiu et al. 1978 |
| *S. typhimurium* (TA98, TA1538, TA1535, TA1537, TA100) | Gene mutation | No data | + | Shimizu et al. 1983 |
| *S. typhimurium* (TA98, TA100) | Gene mutation | + | + | Melnikow et al. 1981 |
| *S. typhimurium* (TA1535, TA1537, TA100NR3) | Gene mutation | – | – | Spanggord et al. 1982b |
| *S. typhimurium* (TA1538, TA98, TA100) | Gene mutation | + | + | Spanggord et al. 1982b |
| *S. typhimurium* (TA1535, TA1538, TA98, TA100) | Gene mutation | – | No data | Anderson and Styles 1978[b] |
| *S. typhimurium* (TA98) | Gene mutation | No data | + | Furukawa et al. 1985 |
| *S. typhimurium* (TA1538, TA98, TA100) | Gene mutation | +[a] | + | McGregor et al. 1980 |
| *S. typhimurium* (TA1537) | Gene mutation | (+) | (+) | McGregor et al. 1980 |
| *S. typhimurium* (TA1535) | Gene mutation | – | – | McGregor et al. 1980 |
| *S. typhimurium* (TA100, D3052, TA1538, TA98) | Gene mutation | No data | + | Probst et al. 1981 |
| *S. typhimurium* (G46, TA1535, C3076, TA1537) | Gene mutation | No data | – | Probst et al. 1981 |
| *Escherichia coli* (WP2, WP2, uvrA) | Gene mutation | No data | – | Probst et al. 1981 |

## TABLE 2-2. Genotoxicity of 1,3-DNB *In Vitro* (continued)

| Species (test system) | End point | Results: With activation | Results: Without activation | Reference |
|---|---|---|---|---|
| Eukaryotic organisms: | | | | |
| Fungi: | | | | |
| *Saccharomyces cerevisiae* | Mitotic recombination | – | – | McGregor et al. 1980 |
| Mammalian cells: | | | | |
| Rat (liver cells) | DNA damage | – | NA | Probst et al. 1981 |

[a]The presence of metabolic activators lessened the toxicity.
[b]Study did not specify which positional isomer of 1,3-DNB was used.

DNA = deoxyribonucleic acid; NA = not applicable; – = negative result; + = positive result; (+) = weakly positive result

## TABLE 2-3. Genotoxicity of 1,3,5-TNB *In Vitro*

| Species (test system) | End point | Results | | Reference |
|---|---|---|---|---|
| | | With activation | Without activation | |
| Prokaryotic organisms: | | | | |
| *Salmonella typhimurium* (TA 1535, TA1537, TA1538, TA100, TA98) | Gene mutation | +[a] | + | McGregor et al. 1980 |
| *S. typhimurium* (TA 1535) | Gene mutation | + | – | Spanggord et al. 1982b |
| *S. typhimurium* (TA 1537, TA 1538) | Gene mutation | – | + | Spanggord et al. 1982b |
| *S. typhimurium* (TA 98, TA100) | Gene mutation | + | + | Spanggord et al. 1982b |
| *S. typhimurium* (TA100NR3) | Gene mutation | – | – | Spanggord et al. 1982b |
| Eukaryotic organisms: | | | | |
| Fungi: | | | | |
| *Saccharomyces cerevisiae* | Mitotic recombination | – | – | McGregor et al. 1980 |

[a]The presence of metabolic activators lessened the toxicity.

– = negative result; + = positive result

## 2.5 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).

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 body by the time biologic 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 1,3-DNB and 1,3,5-TNB are discussed in Section 2.5.1.

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 often not substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by 1,3-DNB and 1,3,5-TNB are discusskd in Section 2.5.2.

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, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, Populations That Are Unusually Susceptible.

### 2.5.1 Biomarkers Used to Identify or Quantify Exposure to 1,3-DNB and 1,3,5-TNB

Very few methods are available for determining the level of 1,3-DNB and its metabolites in human blood and urine (for more information see Chapter 6). Because 1,3-DNB is rapidly absorbed, metabolized, and excreted, measurement of blood levels of this substance or its metabolites 4s limited to exposures of a very large magnitude and that occur within a few, hours of the time at which the blood sample is obtained. Results from an *in vitro* study in rat hepatocytes and microsomes show that the relative rate of conversion of 1,3-DNB to nitroaniline is 7 and 12 minutes, respectively (Cossum and Rickert 1985). This would make it difficult to accurately determine the level of 1,3-DNB in the blood and use it as a marker of exposure.

Very little information is available about the nature of urinary metabolites of 1,3-DNB in humans. In a study that evaluated 1,3-DNB urinary metabolites after a single dermal exposure, amino and nitro metabolites were grouped together and reported as a single value relative to the level of 2,4-dinitrophenol as a standard (Ishihara et al. 1976). Amino and nitro metabolites may be derived from a variety of nitroaromatic compounds; thus, they are not specific for 1,3-DNB.

In rats, 1,3,5-TNB was found to form adducts with blood proteins such as albumin, globulin, and globin, and with DNA from tissues, and it was suggested that these adducts may be useful as markers for exposure to 1,3,5-TNB (Reddy et .al. 1991). Adducts with albumin and globulin reached a maximum one day after a single oral dose, and by day 7 had almost completely disappeared. Adducts with globin peaked by day 2, and after 28 days, 20% of the adducts remained. DNA adducts were also formed in the spleen, liver and stomach. In the spleen, 100% of the adducts were retained for at least 28 days after dosing.

### 2.5.2 Biomarkers Used to Characterize Effects Caused by 1,3-DNB and 1,3,5-TNB

One of the earliest effects of 1,3-DNB exposure is induction of methemoglobin formation. The level of methemoglobin in the blood can be used as an indicator of exposure to 1,3-DNB.

There are two questions to be answered in relation to the specificity of methemoglobin formation as a biomarker for exposure to 1,3-DNB. One pertains to the nature of the reaction and whether 1,3-DNB itself or one of its metabolic intermediaries cause methemoglobin formation. The second question relates to the specificity of the reaction, namely the fact that other nitrobenzene compounds and dinitrobenzene isomers may also cause methemoglobin formation. Methemoglobin formation is a common response to exposure to organic nitrates and is not specific for either 1,3-DNB or 1,3,5-TNB. Once these two issues are resolved, it might be possible to select a more specific biomarker for 1,3-DNB exposure. In the meantime, the levels reflected in a complete blood count can be used as a nonspecific biomarker. These are red cell count, hemoglobin concentration, hematocrit, white cell count, and a peripheral blood smear for cell morphology. They are rapid, relatively inexpensive, and useful for monitoring cohorts of persons possibly exposed to particular members of the nitrobenzene class of chemicals.

Another early symptom of exposure to 1,3-DNB is cyanosis due to oxygen deprivation because of the presence of methemoglobin in the blood. These changes are also not specific for 1,3-DNB and may be produced by other nitrobenzene compounds and dinitrobenzene isomers. Therefore, cyanosis is not a good biomarker for 1,3-DNB exposure.

Although little information is available regarding neurotoxicity of 1,3-DNB, slight headache, nausea, dizziness, and general malaise can accompany exposure to 1,3-DNB (Ishihara et al. 1976; Okubo and Shigeta 1982). These symptoms can occur early or be concomitant with cyanosis, but the correlation between them was not investigated. These symptoms are not specific to 1,3-DNB exposure and therefore are not good biomarkers for 1,3-DNB exposure.

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

## 2.6 INTERACTIONS WITH OTHER CHEMICALS

Limited information is available regarding the influence of other chemicals on the toxicity of 1,3-DNB or 1,3,5-TNB. One study reported that a chemical mixture containing 1,3-DNB was not toxic and did not induce methemoglobin formation. The mixture contained 1,3-DNB (0.5%), ethylene glycol (77.5%

or less), and diethylene glycol (15% or more) (Ishihara and Ikeda 1979). As the mixture was made more polar either by adding water or short-chain dicarboxylic acids, methemoglobin formation was favored. The specific mechanism of this interaction is not known,

## 2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to 1,3-DNB/1,3,5-TNB than will most persons exposed to the same level of 1,3-DNB and 1,3,5-TNB in the environment. Reasons include genetic make-up, developmental. stage, health and nutritional status, and chemical exposure history. These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic and renal) or the pre-existing compromised function of target organs. For these reasons we expect the elderly with declining organ function and the youngest of the population with . immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

No information was located on populations unusually susceptible to toxic effects of 1,3,5-TNB.

In. the review of the literature regarding toxic effects of 1,3-DNB, no information on any population that might be unusually sensitive to 1,3-DNB was found. However, populations that may show increased sensitivity include very young children who have an immature hepatic detoxification system (and less efficient fetal hemoglobin), individuals with impaired liver or kidney function, and those persons who are prone to anemia or are anemic. Also at risk of potential 1,3-DNB toxicity are infants with low levels of nicotinamide adenine dinucleotide diaphorase (enzyme that reduces methemoglobin) or persons congenitally deficient in this enzyme. At increased risk for induction of methemoglobinemia due to exposure to the nitrobenzene class of chemicals may be individuals with such genetic traits as glucose 6 phosphate deficiency, sickle cell trait, or genetically induced unstable hemoglobin forms.

## 2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 1,3-DNB or 1,3,5-TNB. 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 1,3-DNB or 1,3,5-TNB. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

No studies were located regarding the reduction of toxic effects of 1,3,5-TNB. In the two occupational studies on human dermal exposure to 1,3-DNB, no treatment was described to diminish or alleviate 1,3-DNB toxicity (Ishihara and Ikeda 1979; Ishihara et al. 1976). In both studies, affected workers were removed from the 1,3-DNB source and recovered without treatment within 40 days (Ishihara et al. 1976).

### 2.8.1 Reducing Peak Absorption Following Exposure

In order to reduce absorption of I,3-DNB or 1,3,5-TNB following inhalation exposure, patients should be moved to fresh air (HSDB 1994). Following recent ingestion of a substantial amount of either chemical, emesis may be indicated unless the patient is obtunded, comatose, or convulsing (HSDB 1994). Administration of a charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, has also been recommended (HSDB 1994). Following dermal exposure, it is recommended that the exposed area be washed extremely thoroughly with soap and water (HSDB 1994). Eye contamination should be treated by irrigating with copious amounts of tepid water for at least 15 minutes (HSDB 1994).

### 2.8.2 Reducing Body Burden

No studies were located regarding reducing body burden following exposure to 1,3-DNB or 1,3,5-TNB.

### 2.8.3 Interfering with the Mechanism of Action for Toxic Effects

No agents are known to interfere with 1,3-DNB or 1,3,5-TNB cyanosis (resulting from methemoglobin production), but procedures are available and have been recommended to counteract these effects. Cyanosis may be treated with high flow (100%) oxygen administration to saturate all remaining normal hemoglobin with oxygen (Donovan 1990; Ellenhorn and Barceloux 1988). Elevated levels of methemoglobin may be decreased by enhancing the rate of conversion of methemoglobin to hemoglobin. Methylene blue is the antidote of choice in this situation. Ascorbate has been suggested

as an alternative reducing agent, but it is believed to have limited efficacy (Donovan 1990; Ellenhorn and Barceloux 1988). Methylene blue is administered intravenously. It is first reduced to leukomethylene blue by NADPH-dependent methemoglobin reductase in the red blood cell. The leukomethylene blue then acts as an electron donor to reduce methemoglobin to hemoglobin nonenzymatically. Use of methylene blue is generally indicated when methemoglobin levels exceed 30% but may be used at lower methemoglobin levels in persons with pulmonary or cardiovascular disease or with preexisting anemia (Donovan 1990; Ellenhom and Barceloux 1988; Goldfrank et al. 1990). Methylene blue is ineffective in persons with glucose-6-phosphate dehydrogenase deficiency and of limited effectiveness in persons with NADPH-dependent methemoglobin reductase deficiencies (Donovan 1990; Ellenhom and Barceloux 1988; Goldfrank et al. 1990). Severe hemolytic anemia may develop if methylene blue is given to persons with glucose-6-phosphate dehydrogenase deficiency. Caution should also be used when administering methylene blue to others because high doses (>7 mg/kg) may increase methemoglobin levels and cause hemolysis (Donovan 1990; Ellenhom and Barceloux 1988). In cases of failure of methylene blue therapy, exchange transfusions have been used to replace hemoglobin and remove the absorbed toxin (Donovan 1990; Ellenhom and Barceloux 1988).

If seizures develop following exposure to 1,3-DNB or 1,3,5-TNB, administration of diazepam IV bolus has been suggested. Administration of phenytoin is recommended if seizures are uncontrollable or recur (HSDB 1994).

## 2.9 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 1,3-DNB and 1,3,5-TNB 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 1,3-DNB and 1,3,5-TNB.

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 or eliminate the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the

identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

### 2.9.1 Existing Information on Health Effects of 1,3-DNB and 1,3,5-TNB

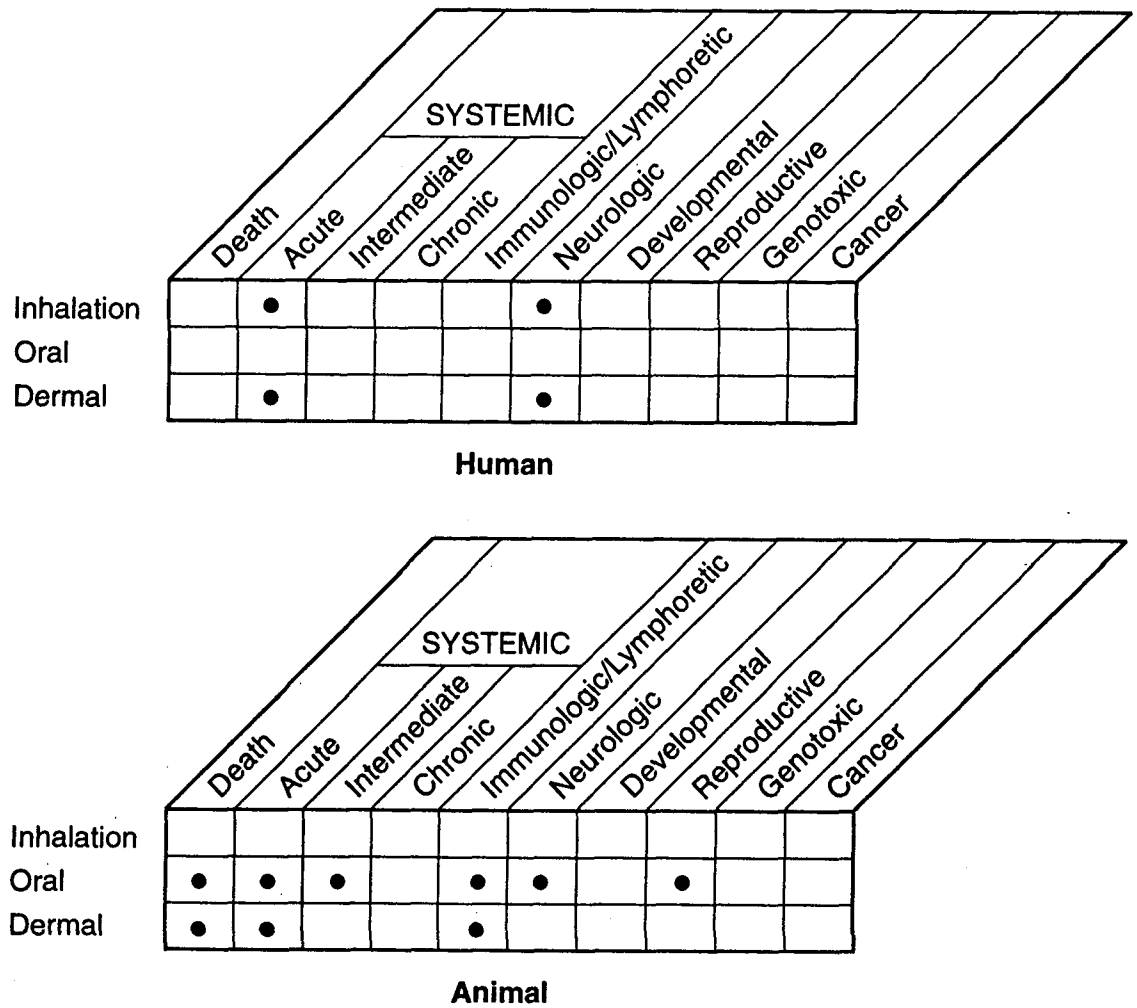
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 1,3-DNB and 1,3,5-TNB are summarized in Figure 2-3 and 2-4, respectively. The purpose of these figures is to illustrate the existing information concerning the health effects of 1,3-DNB and 1,3,5-TNB. 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 1989e), 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. In addition to this information, a database is available through the National Institutes of Health on the metabolism and toxicity of 1,3-DNB.

No studies were located concerning the health effects of 1,3,5-TNB in humans. Information on effects in animals was limited to acute oral and dermal data.

With regard to human health effects of 1,3-DNB, the few available studies involved acute-duration occupational exposure to 1,3-DNB by the inhalation and dermal routes, a case of accidental ingestion of a nitrobenzene dye, and a case of an experimenter who self-applied 1,3-DNB dermally for research purposes. No information was located on intermediate- or chronic-duration exposures in humans by any route. No information is available regarding immunologic, developmental, reproductive, genotoxic, or cancer effects in humans by any route of exposure.

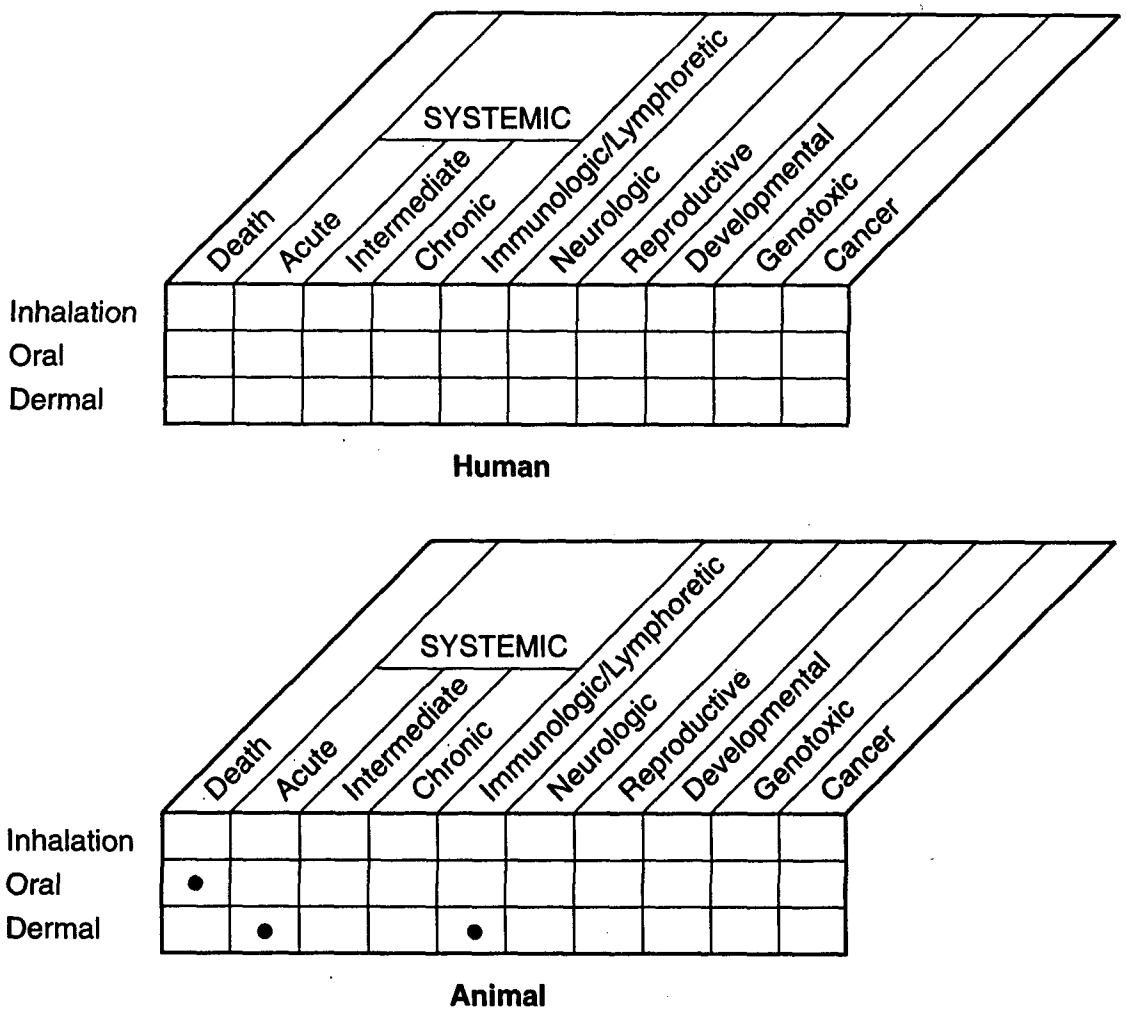
Virtually all of the data regarding the health effects of 1,3-DNB in animals were obtained from studies in which 1,3-DNB was administered orally. No information is available concerning health effects in animals following inhalation exposure, and only two reports on dermal exposure to 1,3-DNB were located. Therefore, information on those two routes of exposure would be useful because of the

**FIGURE 2-3. Existing Information on Health Effects of 1,3-DNB**




● Existing Studies

**FIGURE 2-4. Existing Information on Health Effects of 1,3,5-TNB**




● Existing Studies

potential exposure via those two routes for humans living near ammunition plants and workers in the dyestuffs, plastics, rubber, and other industries.

### 2.9.2 Identification of Data Needs

**Acute-Duration Exposure.** Populations living in the vicinity of ammunition plants may be exposed to 1,3-DNB or 1,3,5-TNB for a short time. Exposure would probably occur via the oral route, but inhalation and dermal exposures cannot be excluded.

No information regarding health effects of 1,3,5-TNB administered by any route is available in humans. Data presented in abstract form provided limited information on oral and dermal toxicity in animals (Desai et al. 1991). Therefore, studies addressing toxic effects of 1,3,5-TNB in animals after acute oral exposure (since this is the most likely route of exposure for human populations in the vicinity of ammunition plants) would provide needed information for estimation of possible 1,3,5-TNB toxicity in humans. Also needed are acute exposure studies of 1,3,5-TNB after inhalation and dermal exposures because exposure by these routes may occur in spite of the low volatility of these compounds.

The hematological system is the major target of 1,3-DNB toxicity in humans and animals following acute exposure by any route. Biochemical changes that occur in blood are responsible for methemoglobin formation leading to oxygen deprivation in the tissues (Donovan 1990; Ellenhorn and Barceloux 1988). That change further results in cyanosis (Linder et al. 1988, 1990; Philbert et al. 1987b; Reader et al. 1991). Studies in animals further indicate that adverse neurological effects such as ataxia (Cody et al. 1981; Linder et al. 1988, 1990; Philbert et al. 1987b), and reproductive effects, such as altered spermatogenesis and infertility (Cody et al. 1981; Hess et al. 1988; Linder et al. 1988, 1990; Moore et al. 1992; Reader et al. 1991) occur after acute oral exposure to 1,3-DNB. These potential targets have not been studied in humans. An acute oral MRL was derived for 1,3-DNB based on adverse reproductive effects in male rats (Linder et al. 1990) (see Section 2.4).

Respiratory changes have not been studied in either humans or animals after acute inhalation exposure to 1,3-DNB. However, slight dyspnea was observed in humans following an acute inhalation exposure to 1,3-DNB (Okubo and Shigeta 1982). Although the volatility of 1,3-DNB is low and thus the levels in the atmosphere are expected to be low, lung absorption of 1,3-DNB is possible in areas close to

ammunition plants and in occupationally exposed workers. It would therefore be useful to perform studies that examine the respiratory effects after acute inhalation exposure to 1,3-DNB. Limited data indicate that humans can absorb 1,3-DNB through the skin (Ishihara et al. 1976; White and Hay 1901), and although.there was no evidence of adverse respiratory effects, well-conducted studies in animals may provide valuable supportive information.

**Intermediate-Duration Exposure.** No studies were located on intermediate-duration exposure to 1,3,5-TNB in humans or animals by any route. Therefore, studies in animals would provide useful information. There were also no studies on intermediate-duration exposure by any route to 1,3-DNB in humans. Studies in laboratory animals following intermediate-duration oral exposure to 1,3-DNB showed that a major target is the hematological system (see Section 2.2.2.2), but other targets include the central nervous system (Cody et al. 1981; Linder et al. 1986) (see Section 2.2.2.4) and the male reproductive system (Cody et al. 1981; Linder et al. 1986) (see Section 2.2.2.5). An intermediate oral MRL was derived for 1,3-DNB based on hematological effects in rats exposed for 12 weeks (Linder et al. 1986) (see Section 2.4). Studies to determine whether adverse effects occur in animals after inhalation or dermal intermediate duration exposure would be useful.

**Chronic-Duration Exposure and Cancer.** No studies were located following chronic-duration exposure to either 1,3-DNB or 1,3,5-TNB in humans or animals. This may be because both compounds were shown to be potent acute toxicants. Animal studies that examine the effects of 1,3-DNB and 1,3,5-TNB after low-level chronic exposure by oral, dermal, and inhalation routes would be of value to determine whether exposures via these routes could cause toxicity in populations living in the vicinity of ammunition plants or in those exposed in industries where these chemical are used.

Because of the lack of data on the carcinogenicity of 1,3-DNB and 1,3,5-TNB, and in the absence of data to adequately describe the mechanism of action, these two compounds are not presently classified as carcinogens. Studies to determine if these two compounds have carcinogenic potential via inhalation, oral and dermal exposure routes would be useful.

**Genotoxicity.** No human or animal in viva studies on the genotoxicity of I,3-DNB or 1,3,5-TNB were located. However, several bacterial mutagenicity studies were located for both chemicals. Depending on the strain of S. typhimurium used in mutagenicity, testing both compounds caused nonsignificant frameshift mutations and base-pair substitutions (Chiu et al. 1978; Furukawa et al. 1985;

Gamer and Nutman 1977; Kaden et al. 1979; Melnikow et al. 1981; Spanggord et al. 1982b) indicating that both 1,3-DNB and 1,3,5-TNB have a mutagenic potential. The results of several studies have also suggested a link between the mutagenicity of 1,3-DNB/1,3,5-TNB and nitroreduction (Chiu et a1.1978; Kerklaan et al. 1987; Probst et al. 1981; Shimizu et al. 1983; Spanggord et al. 1982b). *S: typhimurium* strains deficient in enzyme nitroreductase had nonsignificant gene mutations after exposure to either 1,3-DNB or 1,3,5-TNB (Spanggord et al. 1982b). Further investigation into this link using mammalian cells/systems would be helpful in establishing if the same processes also occur in mammalian cells.

**Reproductive Toxicity.** No studies were found describing reproductive effects of 1,3-DNB in humans and 1,3,5-TNB in humans and animals. Studies in laboratory animals exposed orally to 1,3-DNB show that 1,3-DNB is a potent testicular toxicant (see Section 2.2.2.5) (Blackburn et al. 1988; Cody et al. 1981; Evenson et al. 1989b; Hess et al. 1988; Linder et al. 1986, 1988; Reader et al. 1991). An acute-duration oral MRL was derived on the study by Linder et al. (1990) (see Section 2.4). No studies in animals were found regarding the reproductive effects of exposure to 1,3-DNB by inhalation or dermal routes. Therefore, studies examining the effects on reproduction (including exposure of females during gestation) following inhalation, oral, and dermal exposure to 1,3-DNB would be useful, since oral exposure is the most likely route for people living near ammunition plants, but inhalation and dermal exposure may be more relevant in industrial settings. Animal studies following exposure to 1,3,5-TNB by any of the three routes would be useful to establish if there is potential for reproductive toxicity in people living close to ammunition plants or in occupationally exposed workers.

**Developmental Toxicity.** No human or animal studies on the developmental effects of 1,3-DNB or 1,3,5-TNB for any exposure route were located in this literature review. Animal studies examining postnatal survival and developmental effects following maternal exposure by all routes of exposure would be helpful since potential oral exposure exists for populations living near ammunition plants, and inhalation and dermal exposure may occur in industries involved in dyestuff, plastics, and rubber production.

**Immunotoxicity.** No information on immunotoxicity after exposure to 1,3,5-TNB by any of the three routes is available in humans or animals. Therefore, animal studies following acute, intermediate, and chronic exposure to 1,3,5-TNB via all three routes would help in estimating the

potential immunotoxic effects in humans. Spleen enlargement was reported in acute- (Blackburn et al. 1988) and intermediate-duration (Cody et al. 1981; Linder et al. 1986) studies in animals. These effects, however, were secondary to adverse hematological effects. Studies in laboratory animals following acute exposure to 1,3-DNB by the oral route would help define possible effects on antibody production and cellular immunity. This information could be used to determine populations sensitive to possible exposure to 1,3-DNB at locations close to ammunition plants or in specific workplaces.

**Neurotoxicity.** The few human studies available on dermal and inhalation exposure to 1,3-DNB indicate that the central nervous system. may be a target of 1,3-DNB toxicity (Ishihara et al. 1976; Okubo and Shigeta 1982) (see Sections 2.2.1.4 and 2.2.3.4). Studies in animals support this finding although results in animal studies were mostly obtained after acute oral exposure to 1,3-DNB (Linder et al. 1988, 1990; Philbert et al. 1987b). The animal data have also shown that the severity of neurotoxic effects is dose dependent and that neurotoxicity is probably due to decreased oxygenation because of increased levels of methemoglobin (Cody et al. 1981; Linder et al. 1988; Philbert et al. 1987b). Laboratory animal studies that focus on subtle neurological effects following acute, intermediate, or chronic exposure to a range of doses via oral, inhalation, and dermal routes would help to better estimate potential neurotoxic effects in humans living near ammunition plants and in workers who might be exposed in certain occupational settings.

No studies were found describing neurotoxicity of 1,3,5-TNB in humans or animals. Therefore, studies following oral, inhalation, or dermal exposure to 1,3,5-TNB would be very helpful in evaluating potential neurotoxic effects close to ammunition plants.

**Epidemiological and Human Dosimetry Studies.** No epidemiological studies on exposure to either 1,3-DNB or 1,3,5-TNB have been located. Studies of worker populations and populations living near ammunition plants might be useful to determine effects of low-level acute, intermediate, or possibly chronic exposure to 1,3-DNB or 1,3,5-TNB. If such populations are identified, carefully designed information gathering of immunologic, reproductive, hematologic, neurotoxic, genotoxic, developmental, and carcinogenic effects of the two compounds should be implemented. The correlation of these effects with the levels of methemoglobin associated with exposure would provide useful information regarding potential exposure of populations living near ammunition plants and occupationally exposed workers.

**Biomarkers of Exposure and Effect.**

***Exposure.*** Exposure to 1,3-DNB is currently measured indirectly by determining levels of methemoglobin in the blood (Donovan 1990). However, increased methemoglobin formation is not a specific response to 1,3-DNB exposure and may occur after exposure to other nitrobenzene compounds such as the other two isomers of dinitrobenzene. Determination of methemoglobin levels is widely used and is a reliable detection method. Very few methods are available for direct evaluation of 1,3-DNB levels, and they are not extensively used, probably because of the relatively rapid rate of conversion of 1,3-DNB to its degradation products (Cossum and Rickert 1985). Preliminary data suggested that the formation of adducts of 1,3,5-TNB with tissue DNA and/or with blood proteins may be useful as markers for exposure to 1,3,5-TNB (Reddy et al. 1991). Further research with both 1,3-DNB and 1,3,5-TNB in the area of adduct formation could provide valuable additional information.

***Effect.*** Cyanosis is also an early symptom of exposure to 1,3-DNB (Okubo and Shigeta 1982) and is a result of oxygen deprivation due to the presence of methemoglobin in the blood. However, it is not specific and may occur after exposure to other nitrobenzene compounds or other non-related chemicals.

Nitroaniline is one of the 1,3-DNB metabolites and animal studies designed to evaluate its level in the urine would give information about the usefulness of nitroaniline as a biomarker of 1,3-DNB exposure.

No studies in humans or animals were located dealing with biomarkers of effects after exposure to 1,3,5-TNB. Therefore, research efforts aimed to identifying such a biomarker would be useful.

**Absorption, Distribution, Metabolism, and Excretion.** The few studies available in humans indicate that 1,3-DNB can be readily and rapidly absorbed via the dermal and inhalation routes (Ishihara et al. 1976; Okubo and Shigeta 1982). Quantitative information on the rates of absorption of 1,3-DNB in humans and animals following all routes of exposure are limited (see Section 2.3.1). Obtaining additional quantitative data in animals via all exposure routes and using different vehicles would be helpful for estimating absorption in humans.

No studies were located regarding distribution following inhalation exposure to 1,3-DNB and 1,3,5-TNB in humans and animals. Data on distribution via the dermal and oral routes for humans were not located. There is limited information describing distribution following acute oral exposure to 1,3-DNB in animals. Studies indicate that 1,3-DNB is distributed in the blood, liver, kidneys, and fat tissue (Philbert et al. 1987b; Parke 1961). Additional studies regarding acute oral and dermal exposures would help elucidate the distribution pattern of 1,3-DNB. The oral route is the most likely route of exposure near ammunition plants, while workers in the plants would most likely be exposed by inhalation of dusts and through dermal contact. Only one study was located that provided information regarding distribution of 1,3,5-TNB in animals after acute oral exposure (Reddy et al. 1991). Further studies via all three routes of exposure would be valuable to determine the distribution pattern for 1,3,5-TNB.

No information was located regarding metabolism of 1,3-DNB in humans and animals following inhalation exposure. Information on metabolism following oral exposure is available (see Section 2.3.3.2); however, more information would be useful because the potential exists for exposure to occur in humans via this route.

No studies were located on absorption or metabolism following oral inhalation or dermal exposure to 1,3,5-TNB in humans or animals. Therefore, animal studies are needed to elucidate the absorption process and metabolic path following exposure to 1,3,5-TNB.

No information was located regarding excretion in humans or animals following inhalation or dermal exposure to 1,3-DNB or following exposure to 1,3,5-TNB by any route. Studies in these areas would provide useful information since exposure to these compounds can occur by all three routes. Several studies in animals describe excretion following oral exposure to 1,3-DNB (see Section 2.3.4.2). These studies show quantitatively that the metabolites (2,4-diaminophenol, 1,3-phenylenediamine, 1,3-nitroaniline, and 2-amino-4-nitrophenol) (Parke 1961) are excreted primarily in the urine. Differences in excretion of metabolites have been observed in several species following oral exposure. It is not clear which species is best for determining excretion patterns in humans. Therefore, studies to determine which is the best animal model to be used for extrapolation of data on distribution and excretion patterns of 1,3-DNB to humans would be useful.

**Comparative Toxicokinetics.** Several studies using different animal species (rat, hamster, rabbit) indicate that the kinetics of 1,3-DNB differ across species (McEuen and Miller 1991; Parke 1961; Watanabe et al. 1976). The differences are primarily quantitative. On the basis of kinetic data alone, it is not possible to identify common target organs, but distribution data and toxicity data after oral exposure together suggest similar target/systems organs (hematological and reproductive systems, liver and kidneys. Interspecies differences between rats and hamsters include metabolism and excretion (McEuen and Miller 1991). The interspecies differences, and the lack of data across different routes, point to the possible problem in comparing the toxicokinetics of 1,3-DNB in animals with that in humans. Additional studies using several species exposed to 1,3-DNB by the oral route would help in determining differences and similarities between humans and animals. Also needed are animal studies on 1,3-DNB toxicokinetics after inhalation and dermal exposures. Since no information was located on toxicokinetics of 1,3,5-TNB after inhalation, oral, or dermal exposures, animal studies addressing these issues would be useful in addressing the data needs.

**Methods for Reducing Toxic Effects.** The most notable clinical sign of exposure to 1,3-DNB (and nitroaromatic compounds in general) is increased formation of methemoglobin that can lead to cyanosis (see Section 2.4). This may occur in humans after inhalation (Okubo and Shigeta 1982), oral (Kumar et al. 1990) or dermal (Ishihara et al. 1976) exposure. Methylene blue is considered the antidote of choice for methemoglobinemia (Donovan 1990; Ellenhorn and Barceloux 1988) and was successfully applied in a case of oral poisoning (Kumar et al. 1990). However, methylene blue is ineffective in populations with certain enzymes deficiencies, or may cause unwanted side effects; therefore, studies aimed at developing alternative antidotes for the treatment of methemoglobinemia would be useful.

### 2.9.3 Ongoing Studies

Ongoing studies regarding the health effects of 1,3-DNB and 1,3,5-TNB were reported in the Federal Research in Progress File (FEDRIP 1994) database. Table 2-4 summarizes the ongoing studies that address the health effects of 1,3-DNB and 1,3,5-TNB.' The table also includes research communicated in recent abstracts.

## TABLE 2-4. Ongoing Studies on 1,3-DNB and 1,3,5-TNB

| Investigator | Affiliation | Research description | Sponsor |
|---|---|---|---|
| M.A. Philbert et al. | Rutgers University, New Brunswick, New Jersey | role of antioxidant vitamins and antioxidant enzymes in the etiology of nitrocompound-induced encephalopathies | NIH |
| M.G. Miller[a] | *University of California,* Davis, California | *Mechanisms of 1,3-DNB testicular toxicity* | *NIEHS* |
| M.G. Miller[a] | University of California, Davis, California | Male reproductive toxicity of environmental chemicals | USDA |
| G. Reddy et al. | U.S. Army Biomedical R&D Laboratory, Frederick Maryland | Effect of 1,3,5-TNB on drug metabolizing enzymes in rats | No data |
| G. Reddy et al. | U.S. Army Biomedical R&D Laboratory, Frederick Maryland | Mechanism of interaction of dinitrobenzenes with hemoglobin | No data |
| D.E. Ray et al. | University of Leicester, Leicester, United Kingdom | Functional modulation of auditory pathway damage induced by 1,3-DNB | No data |
| E.R. Kinkead et al. | Mantech Environmental Technology and Wright-Patterson AFB, Dayton, Ohio | Effects of 1,3,5-TNB on reproduction in rats | No data |

[a]FEDRIP 1994

# 3. CHEMICAL AND PHYSICAL INFORMATION

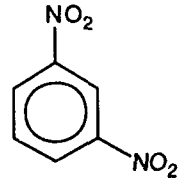
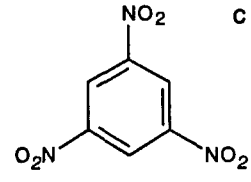
## 3.1 CHEMICAL IDENTITY

Information regarding the chemical identity of 1,3-DNB and 1,3,5-TNB is located in Table 3-l.

## 3.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of 1,3-DNB and 1,3,5-TNB is located in Table 3-2.

## TABLE 3-1. Chemical Identity of 1,3-DNB and 1,3,5-TNB

| Characteristic | 1,3-DNB[a] | 1,3,5-TNB[b] |
|---|---|---|
| Chemical name | 1,3-Dinitrobenzene | 1,3,5-Trinitrobenzene |
| Synonym(s) | m-Dinitrobenzene; 1,3-dinitrobenzol; binitrobenzol; m-DNB; dinitrobenzene | sym-trinitrobenzene; TNB; trinitrobenzene |
| Registered trade name(s) | No data | No data |
| Chemical formula | $C_6H_4N_2O_4$[c] | $C_6H_3N_3O_6$[c] |
| Chemical structure |  d |  c |
| Identification numbers: | | |
| CAS Registry | 99-65-0 | 99-35-4 |
| NIOSH RTECS | CZ7350000 | DC3850000 |
| EPA Hazardous Waste | No data | U234 |
| OHM/TADS | 7800093[e] | 8400321[e] |
| DOT/UN/NA/IMCO | UN1597;IMO 6.1 | UN1354; IMO 4.1; UN0214; IMO 1.1 |
| HSDB | 4017 | 6005 |
| NCI | No data | No data |

[a]Unless otherwise noted, all references for 1,3-DNB are HSDB 1994
[b]Unless otherwise noted, all references for 1,3,5-TNB are HSDB 1994
[c]Merck 1989
[d]Spanggord *et al.* 1982a
[e]OHM/TADS 1991

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 Substance Data Bank from National Library of Medicine; IARC = International Agency for Research on Cancer; 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; STCC = Standard Transport Commodity Code

## TABLE 3-2. Physical and Chemical Properties of 1,3-DNB and 1,3,5-TNB

| Property | 1,3-DNB[a] | 1,3,5-TNB[b] |
|---|---|---|
| Molecular weight | 168.11[c] | 213.11[c] |
| Color | Yellow[d] | Yellow[d] |
| Physical state | Solid[d] | Solid[d] |
| Melting point | 90 °C[c] | 122.5 °C[c] |
| Boiling point | 300–303 °C[c] | 315 °C |
| Density, g/cm$^3$ | 1.575 at 18 °C[c] | 1.76 at 20 °C[e] |
| Odor | No data | No data |
| Odor threshold: | | |
| Air | No data | No data |
| Water | No data | No data |
| Solubility: | | |
| Water at 20 °C | 0.5 g/L[e] | 3.5 g/L[e] |
| Organic solvent(s) | Soluble in chloroform, ethyl acetate, benzene, alcohol[e] | Soluble in benzene, methanol, alcohol, ether and carbon disulfide[e] |
| Partition coefficients: | | |
| Log $K_{ow}$ | 1.49[f] | 1.18[f] |
| Log $K_{oc}$ | 2.33[h,i] | 1.88[g,i] |
| Vapor pressure | | |
| at 20 °C | < 1.0 mm Hg | No data |
| at 25 °C | No data | 3.2x10$^{-6}$ mm Hg[j] |
| Henry's law constant: | | |
| at 20 °C | 2.3x10$^{-6}$ atm-m$^3$/mol[k] | No data |
| at 25 °C | 2.33x10$^{-6}$ atm-m$^3$/mol | 3.08x10$^{-9}$[g] |
| Autoignition temperature | No data | No data |
| Flashpoint | 302 °F | No data |
| Flammability limits | | |
| at 25 °C | No data | No data |
| Conversion factors[i] | 1 ppm = 6.86 mg/m$^3$ | 1 ppm = 8.70 mg/m$^3$ |
| Explosive limits | No data | No data |

[a]Unless otherwise noted, all references for 1,3-DNB are HSDB 1994
[b]Unless otherwise noted, all references for 1,3,5-TNB are HSDB 1994
[c]Lide 1990
[d]Sax and Lewis 1987
[e]Merck 1989
[f]Hennion and Coquart 1993; Murray et al. 1993
[g]DeNeer et al. 1987
[h]Army 1987b
[i]Calculated value
[j]Extrapolated value
[k]EPA 1985a

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

## 4.1 PRODUCTION

1,3-DNB and 1,3,5-TNB have both been prepared by the nitration of benzene with a mixture of nitric acid and sulfuric acid (HSDB 1994). However, I,3-DNB is produced in a two-step nitration process under vigorous conditions, whereas 1,3,5-TNB has been produced from a single-step nitration process with a mixture of fuming nitric acid and fuming sulfuric acid (HSDB 1994; Mark et al. 1978). 1,3-DNB has been synthesized in small quantities as a by-product in the nitration of toluene to form TNT (Mitchell and Dennis 1982).

1,3,5-TNB has also been produced from TNT by removing a methyl group (Sax and Lewis 1987). Trinitrobenzoic acid, the result of oxidation of TNT, has also been decarboxylated to yield 1,3,5-TNB (Merck 1989).

Production volume figures for 1,3-DNB are not easily available because it is produced as a mixture with other nitrobenzene isomers during the manufacturing process. In the United States, DuPont alone reportedly generated 70,000-72,000 pounds of 1,3-DNB annually from production of dinitrobenzene and nitrobenzene (EPA 1991b). The production volumes for 1,3-DNB by other manufacturers are not known. Production data for 1,3,5-TNB by producers in the United States are unknown.

Table 4-1 reports the other facilities, besides DuPont, in the United States that produce and/or process 1,3-dinitrobenzene. The data reported in Table 4-1 are derived from the Toxics Release Inventory (TRI) of EPA (TR192 1994). Only certain types of facilities were required to report to the TRI databank of EPA. Hence, this is not an exhaustive list. The Aldrich Chemical Company (Milwaukee, Wisconsin) and Janssen Chimica (Gardena, California) also produced 1,3-DNB for commercial sale and use (Van et al. 1991). 1,3,5-TNB has been manufactured commercially by Kodak Park Division (Rochester, New York) (OHM/TADS 1991).

Since 1,3,5-TNB releases are not required to be reported under SARA Section 313, there are no data for these compounds in the 1992 Toxics Release Inventory (TR192 1994).

## Table 4-1. Facilities that Manufacture or Process 1,3-DNB

| Facility | Location[a] | Range of maximum amounts on site in pounds | Activities and uses |
|---|---|---|---|
| FIRST CHEMICAL CORP. | PASCAGOULA, MS | 100,000-999,999 | Produce; For on-site use/processing; As a reactant |
| DU PONT CHAMBERS WORKS | DEEPWATER, NJ | 100,000-999,999 | Produce; For on-site use/processing; As a reactant |

Source: TRI92 1994

[a]Post office state abbreviation used

## 4.2 IMPORT/EXPORT

In 1971, an estimated 10,100 pounds of 1,3-DNB were imported into the United States (EPA 1976). However, current import and export data for 1,3-DNB are not available (HSDB 1994). Data for import and export volumes of 1,3,5-TNB are also not available.

## 4.3 USE

Both 1,3-DNB and 1,3,5-TNB have been used for their explosive properties (HSDB 1994). 1,3-DNB has been suggested as a possible substitute for the explosive TNT (HSDB 1994). 1,3-DNB has been used as a camphor substitute in nitrocellulose, a compound used in explosives and propellants (HSDB 1994; Sax and Lewis 1987). 1,3-DNB was manufactured during both world wars as a component in the explosive roburite (EPA 1976). 1,3,5-TNB has been classified as a high explosive and has been used in military and commercial explosive compositions (Merck 1989; Sax and Lewis 1987). A more powerful explosive than TNT, 1,3,5-TNB is less sensitive than TNT to impact (Merck 1989). 1,3,5-TNB has also been used as an explosive for oil wells and mining operations (OHM/TADS 1991).

Commercially, 1,3-DNB has been used extensively as an organic intermediate for *m*-phenylenediamine, a chemical used in the synthesis of aramid fibers and spandex (HSDB 1994). 1,3-DNB is an industrial chemical used in organic synthesis and dyes (McFarlane et al. 1987a). In the medical field, 1,3-DNB has been used as an indicator in the detection of 17-ketosteroids. 1,3-DNB has acted as an electrolytic reducer in the preparation of aminocresols (HSDB 1994). Other uses for 1,3,5-TNB include use as a vulcanizing agent in the processing of natural rubber and as an indicator in acid-base reactions in the pH range of 12.0-14.0 (HSDB 1994).

## 4.4 DISPOSAL

1,3,5-TNB is classified as an EPA hazardous waste and disposal must be carried out according to EPA regulations (HSDB 1994). Wastes generated in the manufacture of explosive components such as 1,3-DNB and 1,3,5-TNB are also characterized as hazardous wastes and EPA regulations for disposal must be followed (EPA 1990a). For more information on the regulations that apply to 1,3-DNB and 1,3,5-TNB, see Chapter 7.

Disposal of both 1,3-DNB and 1,3,5-TNB can be accomplished by high-temperature incineration in a device equipped with an afterburner and a scrubber (HSDB 1994). 1,3-DNB and 1,3,5-77VB have been incinerated by dissolution in a combustible solvent or inert material; the resulting mixture is then sprayed into an incinerator (HSDB 1994; OHM/TADS 1991). 1,3-DNB has also been incinerated after first being wrapped in paper to allow for burning of 1,3-DNB in an unconfined condition (HSDB 1994). 1,3,5-TNB has been classified as a potential candidate for both fluidized bed incineration at temperatures between 450 and 980 °C and rotary kiln incineration at temperatures between 820 and 1,600 °C (EPA 1981). Product residues and sorbent media remaining after high-temperature incineration and scrubbing of 1,35TNB have been packaged in 17H epoxy-lined drums and transported to a RCRA-approved landfill for disposal (OHM/TADS 1991). If appropriate incineration facilities are not available for the disposal of 1,3-DNB, the compound may be buried in a chemical waste landfill, although this practice is not acceptable at municipal sewage treatment plants (OHM/TADS 1991).

Recently investigated methods of treating waste waters contaminated with 1,3-DNB or 1,3,5-TNB and related products include biological treatment, stripping, solvent extraction, and activated carbon adsorption (HSDB 1994).

# 5. POTENTIAL FOR HUMAN EXPOSURE

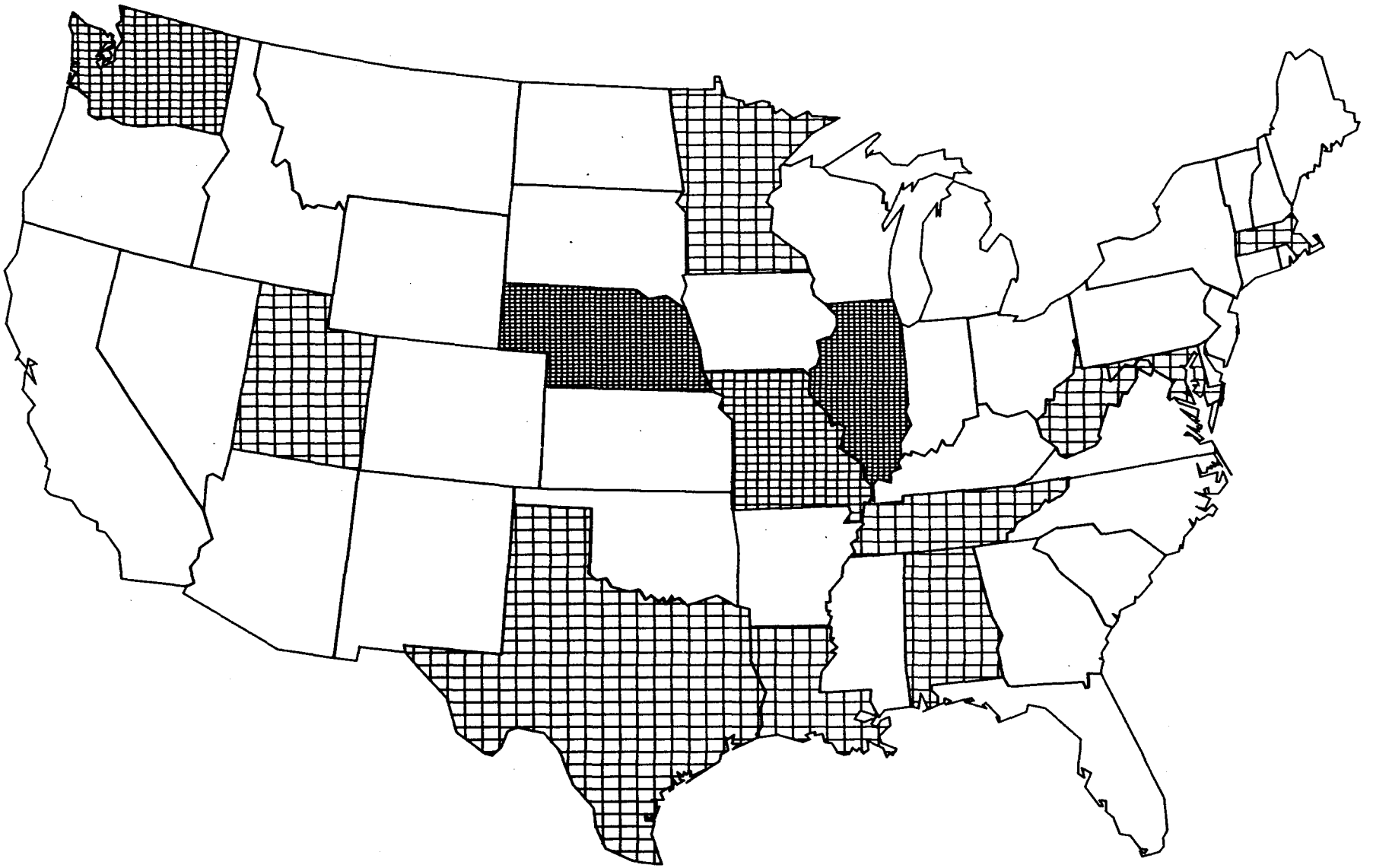
## 5.1 OVERVIEW

The nitroaromatic compounds 1,3-DNB and 1,3,5-TNB are used in the manufacture of explosives. They are formed as by-products during the manufacture of TNT. 1,3-DNB is also used in the manufacture of industrial solvents and dyes. Effluents from Army ammunition plants are primarily responsible for the releases of both compounds into the environment. When released to the air, both compounds have the potential to undergo photolysis. 1,3,5-TNB will also slowly react with photochemically generated hydroxyl radicals, but this is not a significant fate process, No data were located for 1,3-DNB regarding reaction with hydroxyl radicals. Both compounds are mobile in soil and can leach into the groundwater. Hydrolysis is not expected to be an important fate process since aromatic nitro compounds are generally resistant to chemical hydrolysis under environmental conditions. Both compounds are expected to undergo photolysis in water; however, no photolysis data were located for 1,3,5-TNB. The photolytic half-life of 1,3-DNB in water was 23 days. 1,3-DNB can undergo biodegradation under aerobic and anaerobic conditions in water and soil. 1,3,5-TNB is subject to biodegradation under aerobic conditions in water; however, no data were located regarding anaerobic biodegradation in water or anaerobic and aerobic biodegradation in soil. 1,3-DNB and 1,3,5-TNB have been detected in groundwater and soil in the vicinity of Army ammunition plants at levels ranging from ppb to ppm.

The general population is not likely to be exposed to either 1,3-DNB or 1,3,5-TNB. Exposure to both compounds is expected to be limited to areas around Army ammunition plants and other industries, such as dyestuff, and plastic and rubber manufacturing, where these compounds are used. The most likely route of exposure to these compounds is ingestion of contaminated drinking water.

Both 1,3-DNB and 1,3,5-TNB have been identified in 12 and 14 sites, respectively, of the 1,397 hazardous waste sites on the NPL (HazDat 1994). The frequency of these sites within the United States can be seen in Figure 5-1. It should be noted that the number of sites actually tested for 1,3-DNB and 1,3,5-TNB (from the total 1,397 sites) is unknown.

FIGURE 5-1. FREQUENCY OF NPL SITES WITH 1,3-DNB and 1,3,5-TNB CONTAMINATION *




*Derived from HazDat 1994

## 5.2 RELEASES TO THE ENVIROMENT

### 5.2.1 Air

Because 1,3-DNB and 1,3,5-TNB are used in the manufacture of explosives, industrial solvents, plastics, rubber, and dyes, they may be released to air as a result of such uses (Hallas and Alexander 1983). It has been reported by DuPont that approximately 60 pounds of 1,3-DNB are released into the atmosphere annually during processing of the compound (EPA 1991b). According to TR192 (1994) data given in Table 5-1, an estimated total of 1,251 pounds of 1,3-DNB, amounting to 100% of the total environmental release, was discharged to the air from the two manufacturing and processing facilities in the United States in 1992. The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

Since 1,3-DNB has a very low vapor pressure, insignificant amounts would volatilize from water (HSDB 1994; Lyman et al. 1982). Therefore, the amount of 1,3-DNB that might enter the air through evaporation from aquatic effluent streams would be minuscule.

### 5.2.2 Water

Both 1,3-DNB and 1,3,5-TNB are formed as by-products during the manufacture of TNT and can be released to water in discharges from TNT production facilities and munitions plants (ATSDR 1989a, 1989b; Mitchell and Dennis 1982; Spanggord et al. 1982a). 1,3,5-TNB is also formed as a by-product in TNT photolysis (Army 1987b). 1,3-DNB has been detected in the commercial product TNT ‘and can therefore be present in effluents from munitions blending and loading operations (Mitchell and Dennis 1982). 1,3-DNB can also be produced in water by the photoconversion of the munitions by-product 2,4-dinitrotoluene (Higson 1992). 1,3-DNB is one of many nitroaromatic compounds used in the manufacture of dyes and industrial solvents and may be released to water as a result of such uses (Hallas and Alexander 1983). 1,3-DNB can be released to water through wastes and waste discharges from the dye manufacturing process (Dey and Godbole 1986).

## Table 5-1. Releases to the Environment from Facilities that Manufacture or Process 1,3-DNB

| | | | Reported amounts released in pounds per year | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| State[a] | City | Facility | Air | Water | Land | Underground Injection | Total Environment[b] | POTW Transfer | Offsite Waste Transfer |
| MS | PASCAGOULA | FIRST CHEMICAL CORP. | 10 | | | | 10 | | 105 |
| NJ | DEEPWATER | DU PONT CHAMBERS WORKS | 1,241 | | | | 1,241 | | 5 |
| | | Totals | 1,251 | | | | 1,251 | | 110 |

Source: TRI92 1994

[a]Post office state abbreviations used
[b]The sum of all releases of the chemical to air, land, water, and underground injection wells by a given facility
POTW = Publically Owned Treatment Works

### 5.2.3 Soil

Both 1,3-DNB and 1,3,5-TNB may be released to soil in waste discharges from the manufacture of TNT, or from the disposal of TNT wastes, or wastes from munitions plants (Army 1981, 1984b; ATSDR 1987, 1989c; Spalding and Fulton 1988). 1,3-DNB can be released to soil through wastes and waste discharges from the dye manufacturing process (Dey and Godbole 1986).

1,3-DNB and 1,3,5-TNB were not listed in the CLPSD of chemicals detected in soil samples taken at NPL sites only (CLPSD 1989).

## 5.3 ENVIRONMENTAL FATE

### 5.3.1 Transport and Partitioning

The estimated vapor pressure of 1,3-DNB is <l.0 mm Hg at 20 °C (HSDB 1994), indicating that 1,3-DNB will exist entirely in the vapor phase in the atmosphere (Eisenreich et al. 1981). Based on a vapor pressure of $3.2X10^{-6}$ mm Hg at 25°C (see Table 3-2) 1,3,5-TNB is expected to exist partly in the vapor phase and partly in the particulate phase (Eisenreich et al. 1981). The transport of vapor phase 1,3-DNB from the atmosphere to the terrestrial surface is likely to occur mainly by wet deposition, while 1,3,5-TNB is likely to be transported by both wet and dry deposition (Bidelman 1988).

The partitioning of 1,3-DNB in water between water and the suspended solid and sediment has been estimated. Simulation studies based on the octanol-water partition coefficient predict that >99% of 1,3-DNB will remain in the water column and <1% will be adsorbed to suspended solid and sediment (EPA 1991b). 1,3,5-TNB with an octanol-water partition coefficient value lower than 1,3-DNB (see Table 3-2), is expected to exist almost exclusively in the water column.

Henry's law constant for 1,3-DNB was estimated to be $2.33x10^{-6}$ at*m*-$m^3$/mol (HSDB 1994). Based on this value, volatilization from deep quiescent water bodies is expected to be a slow fate process for 1,3-DNB (Lyman et al. 1982). Henry's law constant for 1,3,5-TNB was estimated to be $3.08x10^{-9}$ at*m*-$m^3$/mol at 25 °C using a group structural estimation method (Hine and Mookerjee 1975; HSDB 1994). Based on this value, 1,3,5-TNB is essentially nonvolatile (Lyman et al. 1982). This means

that it is very unlikely that large amounts of either 1,3-DNB or 1,3,5-TNB would be released into the air from contaminated waters.

The soil organic carbon adsorption coefficient ($K_{oc}$) values for 1,3-DNB and 1,3,5-TNB were calculated to be 213.8 and 75.86, respectively (Army 1987b). Based on these $K_{oc}$ values, 1,3-DNB and 1,3,5-TNB are expected to exhibit moderate and high mobility, respectively, in soil; thus, both compounds can leach into groundwater (Swann et al. 1983). However, nitroaromatic compounds show stronger binding toward clay minerals in soil as a result of complex formation between electron donor groups present at mineral surfaces and electron accepting properties of nitroaromatics (Haderlein and Schwarzenbach 1993). This effect is expected to significantly decrease the mobility of such compounds in soil (Haderlein and Schwarzenbach 1993).

The logarithm of the n-octanollwater partition coefficient (log $K_{ow}$) is a useful preliminary indicator of the bioconcentration potential of a compound. The calculated log $K_{ow}$ values for 1,3-DNB and 1,3,5-TNB are 1.52 and 1.18 (Deneer et al. 1987), respectively, suggesting a low potential for .bioaccumulation. An experimental bioconcentration factor (BCF) of 1,3-DNB for the guppy, *Poecilia reticulata,* was reported to be 74.13 (Deneer et al. 1987). This BCF indicates that bioaccumulation in aquatic organisms is not an important fate process. BCF data were not located for 1,3,5-TNB.

The uptake, distribution, and metabolism of 1,3-DNB were studied in hydroponically grown mature soybean plants (McFarlane et al. 1987a). Initial uptake rate constants of 1,3-DNB by soybean plants determined by measuring either chemical loss from solution, $^{14}C$ concentration in plants, or root uptake were similar, ranging from 17 to 22.2 mL/minute. 1,3-DNB remained mostly in the roots, and $^{14}C$ was slowly translocated to the shoots. Examination of $^{14}C$ in the leaves indicated that the translocated chemicals were probably degradation products rather than 1,3-DNB and were metabolized in the roots. The degradation products, however, were not identified (McFarlane et al. 1987a).

No studies were located regarding plant uptake of 1,3,5-TNB.

### 5.3.2 Transformation and Degradation

#### 5.3.2.1 Air

Based on an estimated reaction rate constant of $1.3 \times 10^{-15}$ $cm^3$/mol-second at 25 °C and an average hydroxyl radical concentration of $5.0 \times 10^5$molecule/$cm^3$, the half-life for the reaction of 1,3,5-TNB vapor with photochemically generated hydroxyl radicals in the atmosphere has been estimated to be 34 years (Atkinson 1985, 1987; HSDB 1994). No data were located regarding the photooxidation of 1,3-DNB. Although no data were located regarding direct photolysis of 1,3-DNB or 1,3,5-TNB, both compounds have the potential to photolyze when exposed to sunlight because both can absorb light at wavelengths greater than 290 nm (EPA 1976; Mill and Mabey 1985).

#### 5.3.2.2 Water

No data were located regarding the hydrolysis of 1,3-DNB and 1,3,5-TNB. However, neither compound is expected to undergo hydrolysis since aromatic nitro compounds are generally resistant to chemical hydrolysis under environmental conditions (Lyman et al. 1982). The transformation of 1,3-DNB in water due to reactions with oxidants present in natural bodies of water is not expected to be important in environmental fate processes (EPA 1991b).

If released to water, 1,3-DNB and 1,3,5-TNB may be subject to direct photolysis when exposed to sunlight because both compounds can absorb light at wavelengths greater than 290 nm (EPA 1976; Mill and Mabey 1985). However, no data were located regarding the photolysis of 1,3,5-TNB in water. The photolytic half-life of 1,3-DNB in pure water was calculated to be 23 days (Simmons and Zepp 1986). A three- to four-fold increase in the rate of photoreaction of 1,3-DNB was observed in ambient waters containing natural humic substances or in distilled water containing dissolved humic materials compared to reaction without humic substances (Simmons and Zepp 1986). This enhancement of the reaction rate has been attributed to catalysis of the photoreaction by photosensitization effects of humic substances.

The biodegradation of 1,3-DNB in water requires the presence of microorganisms that are acclimated to 1,3-DNB (EPA 1991b). Therefore, biodegradation of 1,3-DNB is not likely to occur in pristine waters. A mixed bacterial culture, with *Pseudomonas* predominating, adapted to metabolize phenol as

the sole source of carbon had the ability to degrade 100 mg/L 1,3-DNB and 1,3,5-TNB under aerobic conditions (Chambers et al. 1963). Both compounds were slowly oxidized, and 1,3-DNB was degraded at a slower rate than 1,3,5-TNB. Cultures to which 1,3-DNB and 1,3,5-TNB were added had oxygen uptake values ranging from 1.8 to 2.0 times the endogenous rates after 210 and 180 minutes, respectively (Chambers et al. 1963). In this experiment, the resistance of the nitrobenzenes to degradation seemed to decrease as the number of nitro groups on the benzene ring increased (Chambers et al. 1963).

The degradability of 1,3-DNB by microorganisms was studied in environmental samples and in laboratory cultures under aerobic conditions (Mitchell and Dennis 1982). After 21 days of incubation of 5 μg/mL 1,3-DNB with Tennessee River water samples taken downstream from the Volunteer Army Ammunition Plant (a munitions production facility in Chattanooga, Tennessee), the microbially mediated disappearance of 1,3-DNB was complete (Mitchell and Dennis 1982). The results showed that microorganisms from the Tennessee River could be grown on 1,3-DNB as a sole carbon source and could mineralize the compound. The half-life of 1,3-DNB in Tennessee River samples was estimated to be one day, assuming a total of one million total microorganisms per mL and a temperature of 25 °C. The half-life of 1,3-DNB in enrichment cultures grown on 1,3-DNB was 9.7 days. The authors concluded that the more rapid rate of 1,3-DNB removal seen during primary screening (one day) could result from enhanced biodegradation by microorganisms in a more natural state. Results also showed that the enrichment culture was specific for 1,3-DNB. The Tennessee River microorganisms grown on 1,3-DNB did not adapt to metabolize 1,3-TNB (MitcheII and Dennis 1982).

The transformation of 1,3-DNB was measured in sewage sludge effluent maintained for 28 days under aerobic and anaerobic conditions (Hallas and Alexander 1983). Under aerobic conditions, approximately 40% was degraded in 28 days, while under anaerobic conditions, approximately 80% was degraded in 28 days. Nitroaniline was the product formed from 1,3-DNB degradation under both aerobic and anaerobic conditions (Hallas and Alexander 1983).

#### 5.3.2.3 Sediment and Soil

1,3-DNB was biodegraded under aerobic conditions to carbon dioxide by a microbial strain, *Candida pulcherrima***,** isolated from soil contaminated with 1,3-DNB manufacture wastes (Dey and Godbole

1986). Some biotransformation products detected in the metabolic pathway of biodegradation of 1,3-DNB included *m*-nitrophenol, *m*-aminophenol, resorcinol, fumaric acid, and some volatile fatty acids (Dey and Godbole 1986). A pure culture of *Rhodococcus sp.* isolated from soils contaminated with nitroaromatics was capable of using 1,3-DNB as a sole source of nitrogen. This culture metabolized 1,3-DNB to nitrite via a 4-nitrocatechol pathway even in the presence of high amounts of ammonia (Dickel and Knackmuss 1991).

Sixteen microorganisms isolated from soil exposed to the waste water effluent from 1,3-DNB manufacture had the ability to effectively degrade 1,3-DNB in synthetic media and 1,3-DNB waste under aerobic conditions (Dey et al. 1986). The percentage degradation of 1,3-DNB ranged from 32% to 87% and from 35% to 92% under stationary and shake culture conditions respectively, in a synthetic medium. *Streptomyces aminophilus* showed maximum degradation both under shake and stationary culture conditions, followed by *Streptomyces cacaoi, Micromonospora cabali* and *Micrococcus colpogenes.* Data on the percentage degradation of 1,3-DNB, based on the percentage reduction in chemical oxygen demand (COD) of the 1,3-DNB manufacture waste brought about by these organisms, showed that the percentage reduction ranged from 17 to 55% and from 19 to 53% under shake and stationary culture conditions, respectively. *M. colpogenes* gave maximum reduction in COD, followed by *S. aminophilus, S. cacaoi,* and *M. cabali.* The effects of different environmental conditions on 1,3-DNB degradation were also examined (Dey et al. 1986). The isolates were inoculated in a synthetic medium and incubated for 7 days under the following conditions: at different temperatures-22, 26, 37, and 40 “C; at different pH levels, ranging from 5 to 11; at different inoculum densities, from 0.3 to 9 million cells per mL of the medium; and with different periods of incubation at room temperature (26 °C) ranging from 3 to 35 days. Under conditions of varying temperature, results showed that for all microorganisms there was a rise in percentage degradation with a rise in temperature from 22 to 37 °C, followed by a steep fall in percentage degradation with a° urther rise in temperature from 37 to 40° C. Data on the percentage degradation under different pH levels showed that 13 species belonging to the genera *Streptomyces, Micrococcus, Staphylococcus, Micromonospora, Candida, Klebsiella, Vibrio,* and *Aspergillus* showed maximum degradation ranging from 40 to 87% at pH 9. Only three species belonging to the genera *Bacillus* showed maximum degradation ranging from 38 to 47% at pH 8. Of all the species under study, *S. aminophillus* gave maximum degradation at all pH levels, followed by S. *cacaoi* and *M. colpogenes.* Under conditions of different inoculum densities, data showed that three million cells per mL of the medium as the inoculum density was the optimum for maximum degradation of 1,3-DNB. Under conditions of

different incubation periods, data showed that with the longest incubation period (35 days), 100% degradation was exhibited by *S. aminophilus, S. cacaoi, M. caballi, M. colpogenes, Micrococcus roseus, Micrococcus luteus, Staphylococcus saprophyticus,* and *Staphylococcus aureus***.**

No studies were located regarding the transformation and degradation of 1,3,5-TNB in soil.

## 5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

No monitoring studies were located that gave levels of 1,3-DNB or 1,3,5-TNB in air.

### 5.4.2 Water

1,3-DNB and 1,3,5-TNB were detected in effluent (condensate water) from the production and purification of trinitrotoluene (TNT) (Spanggord et al. 1982a). 1,3-DNB was detected in 97.5% of the 54 TNT samples collected over a period of 1 year at concentrations ranging from 0.2 to 8.5 mg/L (ppm) (detection limit not reported). 1,3,5-TNB was detected in 3.8% of the 54 TNT samples at concentrations ranging from 0.06 to 0.20 mg/L (ppm) (Spanggord et al. 1982a).

1,3,5-TNB was detected in water from on-site and off-site wells at maximum concentrations of 352 and 114 ppb, respectively, at the Cornhusker Army Ammunition Plant (CAAP) near Grand Island, Nebraska (ATSDR 1989a). CAAP is an NPL site; CAAP is not currently producing or storing explosive materials. Present activities at the plant are limited to maintenance operations, leasing of property for agriculture and livestock grazing, storage building leasing, and wildlife management (ATSDR 1989a). On-site groundwater sampling at the Milan Army Ammunition Plant (MAAP) in Tennessee identified 1,3,5-TNB at concentrations ranging from non-detectable to 976 ppb (ATSDR 1989c). MAAP is an NPL site. From 1942 to 1978, waste water from a munitions demilitarization process line was discharged into unlined settling ponds (ATSDR 1989c). The Louisiana Army Ammunition Plant (LAAP) is a shell manufacturing and explosives load, assembly, and pack facility (Army 1988). From 1951 to 1980, waste waters were trucked to and discharged into a series of artificial leaching pits. This resulted in contamination of groundwater, soil, and sediments (Army 1988). Detectable levels of 1,3-DNB and 1,3,5-TNB measured in groundwater at LAAP ranged from

1.2 to 195 μg/L (ppb) and from 0.8 to 7,720 μg/L (ppb), respectively (Army 1988). 1,3,5-TNB was also detected in surface water at LAAP at a concentration of 2 ppm (ATSDR 1989b).

### 5.4.3 Sediment and Soil

1,3,5-TNB has been detected in contaminated soil at the Alabama Army Ammunition Plant in Childersburg, Alabama (Army 1981; ATSDR 1987). The contaminants remain from World War II production activities. The levels of 1,3,5-TNB detected in soil were 614 ppb (smokeless powder manufacturing area), <368-2,540 ppb (magazine area), <368-3,920 ppb and 3,920 ppm (flashing ground), and 1,950 ppm (aniline sludge basin) (Army 1981; ATSDR 1987). 1,3,5-TNB was found on-site at the Savanna Army Depot (Illinois) in soil samples at a maximum concentration of 2,770 ppb (ATSDR 1989d). The Savanna Army Depot is an NPL site. It is an Army munitions plant engaged in munitions renovation, loading, demolition, and burning (ATSDR 1989d). 1,3,5-TNB was detected in soil samples collected from the Iowa Army Ammunition Plant in 1983 (Army 1985a; Jenkins and Grant 1987). Mean levels of 1,3,5-TNB in soil taken from an old ordnance-burning area that had not been used since 1981 ranged from 5 1 to 62 μg/g (Army 1985a; Jenkins and Grant 1987). Mean levels of 1,3,5-TNB taken from the surface of an old disposal lagoon ranged from 0.27 to 0.45 μg/g (Army 1985a; Jenkins and Grant 1987). Mean levels of 1,3-DNB detected in field-contaminated soil at an Army installation in Tennessee ranged from 0.77 to 1.5 μg/g (Jenkins et al. 1989).

### 5.4.4 Other Environmental Media

## 5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The general population is not likely to be exposed to 1,3-DNB or 1,3,5-TNB. Exposure is expected to be limited to populations in areas around Army ammunition plants where 1,3-DNB and 1,3,5-TNB have been produced. The most likely route of exposure for populations living in the vicinity of the Army ammunition plants is ingestion of contaminated drinking water. Dermal contact with contaminated soil is also a possible but unlikely route of exposure.

Occupational exposure to 1,3-DNB and 1,3,5-TNB can occur when workers handle the compounds in explosives plants and other industries, such as dyestuffs, plastics, and rubber, that use these compounds during manufacturing processes. The National Occupational Exposure Survey (NOES), conducted by

NIOSH from 1981 to 1983, estimated that 2,489 workers were exposed to 1,3-DNB in 41 businesses and health services (NOES 1991). The workers included in this survey were chemists (except biochemists), geologists, geodesists, clinical laboratory technologists and technicians, and health aides (except nursing).

## 5.6 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Persons living near Army ammunition plants may have a higher risk of exposure to 1,3-DNB and 1,3,5-TNB resulting from ingestion of contaminated drinking water or contact with contaminated soil.

## 5.7 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 1,3-DNB and 1,3,5-TNB is available. Where adequate information is not available, ATSDR, in conjunction with 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 1,3-DNB and 1,3,5-TNB.

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 or eliminate the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

### 5.7.1 Identification of Data Needs

**Physical and Chemical Properties.** The physical and chemical properties of 1,3-DNB and 1,3,5-TNB are sufficiently characterized to permit estimation of their environmental fate (Army 1987b; Merck 1989; DeNeer et al. 1987; HSDB 1994). Therefore, no additional studies are needed at this time.

**Production, Import/Export, Use, and Release and Disposal.** 1,3-DNB has been produced and used commercially in the United States (HSDB 1994). The information that is available on production volumes of 1,3-DNB is neither complete not current. DuPont reportedly generates 70,000-72,000 pounds of 1,3-DNB annually from production of nitro- and dinitro-benzenes (EPA 1991b). The production volumes of 1,3-DNB by other manufacturers are not known. Data on current and past production volumes for 1,3-DNB and 1,3,5-TNB needed for the discussion of production trends of these compounds are not available. In 1971, an estimated 10,100 pounds of 1,3-DNB was imported into the United States (EPA 1976). No data on either the past or current import/export volumes for 1,3,5-TNB were located. Therefore, the history of production and import/export data for both 1,3-DNB and 1,3,5-TNB are needed. Both 1,3-DNB and 1,3,5-TNB have been used for their explosive properties (HSDB 1994). 1,3-DNB has been suggested as a possible substitute for the explosive TNT (HSDB 1994). 1,3,5-TNB has been classified as a high explosive and used in explosive compositions (Merck 1989; Sax and Lewis 1987). Commercially, 1,3-DNB has been used in organic synthesis and dyes (McFarlane et al. 1987a). 1,3,5-TNB has been used as a vulcanizing agent in the processing of natural rubber and as an indicator in acid-base reactions in the pH range of 12.0-14.0 (HSDB 1994). Exposure to these compounds is limited to areas around Army ammunition plants. The most likely route of exposure for populations living near Army ammunition plants is ingestion of contaminated drinking water.

Data on the most commonly used disposal methods are sufficient (EPA 1981; HSDB 1994); however, estimates of, amounts disposed of by each method are needed. 1,3-DNB, 1,3,5-TNB, and the wastes generated in the manufacture of' 1,3-DNB and 1,3,5-TNB are classified as EPA hazardous wastes and disposal must be carried out according to EPA regulations (HSDB 1994).

According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required, to submit chemical release and off-site transfer information to the EPA. The Toxics Release Inventory (TRI), which contains this information for 1992, became available in May of 1994. This database will be updated yearly and should provide a list of industrial production facilities and emissions.

**Environmental Fate.** Based on its low vapor pressure and low value for Henry's law constant (HSDB 1994), it is unlikely that 1,3,5-TNB will partition to the air from soil or natural bodies of water (Lyman et al. 1982). The higher values for vapor pressure and Henry's law constant (HSDB

1994) for 1,3-DNB suggest that it is likely to volatilize from shallow soil or water, but not from deep quiescent water or deep soil (Hine and Mookerjee 1975; Lyman et al. 1982). Both compounds are mobile in soil and can leach into groundwater. 1,3,5-TNB exhibits a higher mobility than 1,3-DNB (Army 1987b; Swann et al. 1983). Neither compound adsorbs to sediments in water to any great extent (Swann et al. 1983). Both compounds have the potential to undergo direct photolysis in air (EPA 1976; Mill and Mabey 1985); however, no direct atmospheric photolysis studies for either compound were located. Therefore, it would be useful to conduct further research to determine the effect of photolysis of these compounds in the vapor phase diluted with air. The reaction rate of 1,3,5-TNB in the presence of photochemically generated hydroxyl radicals is very slow, with an atmospheric half-life of 34 years (Atkinson 1985, 1987; HSDB 1994). No studies were located for 1,3-DNB regarding its reaction with hydroxyl radicals. Hydrolysis is not expected to be an important fate process for either compound (Lyman et al. 1982). Both compounds are expected to undergo photolysis in water; however, no experimental photolysis data were located for 1,3,5-TNB. Experimental data determining the effects of photolysis of 1,3.5-TNB in water would be useful. The experimental photolytic half-life of 1,3-DNB in water is 23 days, and the reaction is sensitized by humic substances (Simmons and Zepp 1986). 1,3-DNB can undergo biodegradation under aerobic and anaerobic conditions in water and soil (Chambers et al. 1963; Mitchell and Dennis 1982). 1,3,5-TNB is subject to biodegradation under aerobic conditions in water (Chambers et al. 1963); however, no data were located regarding anaerobic biodegradation in water or anaerobic and aerobic biodegradation in soil. Further research on the rates of biodegradation of 1,3,5-TNB in water and soil under anaerobic conditions, and of biodegradation of 1,3,5-TNB in soil under aerobic conditions would provide valuable information. Biodegradation half-life data in water and soil are needed for both compounds. This information will be helpful to better identify the most important pathways of human exposure to each compound.

**Bioavailability from Environmental Media.** Limited data indicate that 1,3-DNB is absorbed in humans following inhalation and dermal exposure (Ishihara and Ikeda 1979; Okubo and Shigeta 1982). No studies were located regarding absorption of 1,3-DNB following oral exposure. No studies were located regarding absorption of 1,3,5-TNB following inhalation, oral, or dermal exposure. More information regarding all absorption routes for both compounds, particularly on absorption following ingestion of contaminated drinking water and soil or plants grown in contaminated environments, are needed to better characterize the bioavailability of 1,3-DNB and 1,3,5-TNB.

**Food Chain Bioaccumulation.** Based on low log $K_{ow}$ values, both compounds have a low potential for bioaccumulation (Deneer et al. 1987). Based on a low experimental BCF for 1,3-DNB, bioaccumulation in aquatic organisms is not an important fate process (Deneer et al. 1987). No BCF data were located for 1,3,5-TNB. Data indicate that 1,3-DNB bioaccumulates in plants (McFarlane et al. 1987a). No studies were located regarding plant uptake of 1,3,5-TNB. Data are needed regarding the bioconcentration and biomagnification potential of both compounds in terrestrial food chains. This information would be useful to evaluate the importance of accumulation of these compounds in the food chain and on subsequent human exposure to 1,3-DNB and 1,3,5-TNB via the food chain.

**Exposure Levels in Environmental Media.** 1,3-DNB and 1,3,5-TNB were detected in groundwater and soil at Army ammunition plants (Army 1985a, 1988; ATSDR 1987, 1989a, 1989b, 1989c; Jenkins and Grant 1987; Jenkins et al. 1989). Data are needed regarding levels of 1,3-DNB and 1,3,5-TNB in air. No data were located regarding human intake estimates of 1,3-DNB and 1,3,5-TNB. This information would be helpful in evaluating human exposure from each medium. Reliable monitoring data for these compounds in contaminated media at ammunition plants/waste sites are needed so that the information obtained on their levels in the environment, and the resulting body burden caused, can be used to assess the potential risk of adverse health effects in populations living near ammunition plants/waste sites.

**Exposure Levels in Humans.** 1,3-DNB and 1,3,5-TNB have not been detected in human blood, urine, fat, or breast milk; however, 1,3-DNB has been detected in the urine and blood of rodents fed the compound (Bailey et al. 1988; McEuen and Miller 1991; Nystrom and Rickert 1987). Biological monitoring data for both 1,3-DNB and 1,3,5-TNB are needed for populations living near Army ammunition plants and for occupationally exposed populations. This information is necessary for assessing the need to conduct, studies on these populatrons.

**Exposure Registries.** No exposure registries were located for 1,3-DNB and 1,3,5-TNB. This compound is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The compound 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 the exposure to this compound.

### 5.7.2 Ongoing Studies

No ongoing studies were located for either 1,3-DNB or 1,3,5-TNB.

# 6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring 1,3-DNB and 1,3,5-TNB in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify 1,3-DNB and 1,3,5-TNB. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect I,3-DNB and 1,3,5-TNB in environmental samples are the methods approved by federal organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter may be 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 may be included that refine previously. used methods to obtain lower detection limits, and/or to improve accuracy and precision.

## 6.1 BIOLOGICAL SAMPLES

The data on analytical methods for detecting 1,3-DNB and 1,3,5-TNB and their metabolites in biological media are very limited. The few methods that have been used are discussed in the following section and summarized in Table 6-l.

1,3-DNB and its metabolites have been determined in the blood and urine of rodents fed the compound (Bailey et al. 1988; McEuen and Miller 1991; Nystrom and Rickert 1987). The methods to detect 1,3-DNB include high-resolution gas chromatography (HRGC) with electron capture detection (ECD), high-performance liquid chromatography (HPLC) with radioactivity detection (RAD) (for radiolabeled compounds) or ultraviolet (UV) detection or liquid scintillation counting (LSC) (for radiolabeled compounds), gas chromatography (GC) with mass spectrometry (MS), and spectrophotometry. It should be noted that the HPLC/RAD method is not suitable for the determination of 1,3-DNB and its metabolites in humans since it requires exposure to radiolabeled compounds. A reported method for quantitating 1,3-DNB and its metabolites in blood and urine by HRGC/ECD has a limit of detection in the low ppb range, and both recovery (≈110%) and precision (±3% coefficient of variation [CV]) of the method were excellent (Bailey et al. 1988). The reported methods based on HPLC separation and detection/quantification of radioactivity (McEuen and Miller 1991; Nystrom and Rickert 1987) are not suitable for monitoring human exposure because they depend

## TABLE 6-1. Analytical Methods for Determining 1,3-DNB and 1,3,5-TNB in Biological Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Blood (1,3-DNB and metabolites) | Extraction with ethyl acetate; centrifugation | HRGC/ECD | 10 µg/L | 110–111 | Bailey et al. 1988 |
| Blood (1,3-DNB and metabolites) | Extraction with methanol and centrifugation; elution from reverse-phase column with potassium phosphate/methanol | HPLC/UV/LSC | No data | No data | McEuen and Miller 1991 |
| Blood (metabolites) | Extraction with methanol and centrifugation; evaporation; redissolution in water and extraction with ethyl acetate; $H_2O$ removed (anhydrous $Na_2SO_4$) and concentration; separation by TLC | GC/MS | No data | No data | McEuen and Miller 1991 |
| Urine (1,3-DNB and metabolites) | Elution from reverse-phase column with methanol/potassium phosphate/ tetrabutylammonium hydrogen sulfate | HPLC/UV/LSC | No data | No data | McEuen and Miller 1991 |
| Urine (metabolites) | Extraction sample with ethyl acetate; separation by reverse-phase HPLC; derivatization with N-methyl-N-trimethyl-siyltrifluoroacetamide | GC/MS | No data | No data | McEuen and Miller 1991 |
| Urine (1,3-DNB and metabolites) | Centrifugation; elution from reverse-phase columns with sodium phosphate/ acetonitrile | HPLC/RAD<br>GC/MS | No data<br>No data | No data<br>No data | Nystrom and Rickert 1987 |

**TABLE 6-1. Analytical Methods for Determining 1,3-DNB and 1,3,5-TNB in Biological Samples (continued)**

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Urine (NB) | Addition of concentrated HCl and Zn dust; addition of phenol red indicator; pH adjustment to 9.0 with NaOH solution; dilution and centrifugation; removal of supernatant and addition of phosphate buffer; pH adjustment to neutrality; reaction with sodium salt of 1,2-naphthoquinone-4-sulfonic acid; extraction with $CCl_4$; centrifugation; removal and filtration of organic layer | Spectrophotometry | 0.8 mg/L | 62–78 | Dangwal and Jethani 1980 |
| Skin or clothing | Skin wiped with isopropanol swabs or contaminant from clothing transferred by vacuuming onto membrane filters; extraction with acetonitrile and purification by HPLC. | HRGC/TEA | No data | No data | Lloyd 1991 |
| Handswab, standards (1,3-DNB) | Hand wiped with dry swab; extraction with methanol/potassium phosphate | HPLC/EC (PMDE) | 10 pg/inj (standards) | No data | Lloyd 1983 |
| Handswab (1,3,5-TNB) | Hand wiped with swab; swab extraction with methyl *tert*-butyl ether; centrifugation; evaporation of supernatant; redissolution in methyl *tert*-butyl ether in pentane; clean-up on Amberlite XAD-7® column, elution with ethyl acetate; concentrate | HRGC/TEA<br>HRGC/ECD | pg-low ng<br>pg-low ng | No data<br>No data | Douse 1985 |

$CCl_4$ = carbon tetrachloride; EC = electrochemical detection; ECD = electron capture detection; GC = gas chromatography; HCl = hydrogen chloride; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; inj = injection; LSC = liquid scintillation counting; MS = mass spectrometry; NaOH = sodium hydroxide; $Na_2SO_4$ = sodium sulfate; NB = nitrobenzene; PMDE = pendant mercury drop electrode; RAD = radiation absorbed dose; TEA = thermal energy analyzer; TLC = thin layer chromatography; UV = ultraviolet detector; Zn = zinc

on exposure to a radiolabeled dose of 1,3-DNB. GC/MS is a sensitive and highly selective method of detecting 1,3-DNB in blood and urine but has only been used for qualitative confirmation of the compound (McEuen and Miller 1991). It is possible that a modification of the method could be used for quantification of 1,3-DNB and its metabolites in these matrices. A spectrophotometric method for determining nitrobenzene in urine has been developed (Dangwal and Jethani 1980). This method could also be used to determine 1,3-DNB and 1,3,5-TNB in urine because it is somewhat selective for many nitro and amino benzene-based compounds. However, it is not as useful as HRGC/ECD because it is not as selective, and the sensitivity is several orders of magnitude less (ppm). An enzyme-linked immunosorbent assay (ELISA) has been proposed for the determination of 1,3-DNB in biological samples (Miller et al. 1991). The method showed good specificity and comparable recovery of 1,3-DNB from blood samples when compared with HPLC/UV and HPLC/radiochemical detection. No information was located that specifically discussed the detection of 1,3,5-TNB and metabolites in blood and urine by any method.

Methods have been developed for the detection of both 1,3-DNB and 1,3,5-TNB in extracts from hand-swab samples. The methods employ HRGC/ECD, HRGC/thermal energy analyzer (TEA), and HPLC/electrochemical detection (EC). Data are inadequate for a comparison of the sensitivity and reliability of these methods. Both HPLC and HRGC are effective in separating the analyte from other nitro compounds and contaminants (Douse 1985; Lloyd 1983). Detection by TEA (Douse 1985) and EC using a pendant drop mercury electrode (PDME) (Lloyd 1983) is more selective than ECD (Douse 1985). All three detectors were sensitive to ppb levels of analyte. The PDME has a unique advantage in that a new mercury drop can be formed between samples, and therefore, this detector is not subject to degeneration from contamination build-up. This makes the PDME highly reproducible (precision of 1.8% CV).

## 6.2 ENVIRONMENTAL SAMPLES

A large variety of methods has been described for the detection of 1,3-DNB and 1,3,5-TNB in environmental samples. These include GC or HRGC (combined with ECD, TEA, MS, nitrogen-phosphorus detection [NPD], or flame ionization detection [FID]), and HPLC (combined with UV and/or photoconductivity [PC] detection). Other methods that do not need chromatographic separation before quantitation, including MS, cyclic and differential pulse voltammetry, spectrophotometry, and assays based on chemical oxygen demand (COD) and total organic carbon (TOC), have also been used

or tested. Table 6-2 contains a summary of several representative methods for determining 1,3-DNB and 1,3,5-TNB in various environmental media. Methods in which nitrobenzene was the analyte have been included when the methods apply to 1,3-DNB and 1,3,5-TNB as well. For several of the methods included in Table 6-2, nitrobenzene is the analyte being investigated, although the method should also be useful for analysis of 1,3-DNB and/or 1,3,5-TNB (when nitrobenzene data were used, this is indicated in the Sample column).

The few methods located for analysis of nitro compounds in air have not been well characterized for 1,3-DNB or 1,3,5-TNB. Only one method considered the analysis of 1,3-DNB specifically. The remainder were for analysis of nitrobenzene but could also be applied to the analysis of 1,3-DNB or 1,3,5-TNB. Most air samples are preconcentrated by collection on a solid sorbent prior to measurement; however, grab samples or liquid impingers have been used in some methods for the collection of 1,3-DNB. HRGC and GC with FID have been used to measure 1,3-DNB and nitrobenzene in air samples (Andersson et al. 1983; Cooper et al. 1986; Kebbekus and Bozzelli 1982). Under the experimental conditions used, reliability was adequate, with accuracy ranging from 52 to 84% and precisions ranging from 6 to 30% CV (Andersson et al. 1983; Kebbekus and Bozzelli 1982). The lower recoveries and precisions were obtained with nitrobenzene (Kebbekus and Bozzelli 1982) and could be substantially different for 1,3-DNB and 1,3,5-TNB. However, tests with 1,3-DNB and 1,3,5-TNB would have to be conducted to determine reliability parameters for these compounds. Sensitivity for HRGC/FID using nitrobenzene as the analyte was in the low ppt (Kebbekus and Bozzelli 1982). A comparison of HRGC with either NPD or FID showed that NPD was far more selective for nitro compounds than FID (Cooper et al. 1986). The detection limit for HRGC/NPD under the conditions used was in the. low ppb, and the authors could not quantify nitrobenzene using HRGCYFID. A spectrophotometric method has been developed for detection of nitro and amino benzene-based compounds in air (Dangwal 1981). Since the method cannot differentiate between the various nitro- and amino-benzene compounds, it is substantially less selective than other available methods. The sample preparation is more complex than with other tested methods and involves extraction with carbon tetrachloride, a potentially hazardous chemical. In addition, the sensitivity is several orders of magnitude less (ppm) than the’sensitivity of the HRGC and GC methods.

Both GC (high and low resolution) and HPLC may be used to separate nitrobenzene compounds in water. The most common detector for HPLC analysis is UV. For the GC methods, several detectors have been used, including NPD, ECD, FID, TEA, and MS. The sensitivity of the methods varies from

## TABLE 6-2. Analytical Methods for Determining 1,3-DNB and 1,3,5-TNB in Environmental Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Air (1,3-DNB) | Collection on personal sampler containing Amberlite XAD-2; desorption with diethyl ether | GC/FID | No data | 79–84 | Andersson et al. 1983 |
| Air (NB) | Collection on Tenax; thermal desorption | HRGC/FID | 0.01 ppb | 52 | Kebbekus and Bozzelli 1982 |
| Air (NB) | Collection in ethanol; reaction with concentrated HCl and zinc dust; react with sodium salt of 1,2-naphtho-quinone-4 sulfonic acid at pH 8.0; extraction with $CCl_4$; concentration; separation by paper chromatography; extraction of fractions with $CCl_4$ | Spectrophotometry | 10µg | 93 | Dangwal 1981 |
| Gas effluents (NB) | Collection in stainless steel container or on silica gel traps; dilution of canister sample with $N_2$; desorption of silica gel with ethanol | HRGC/FID<br>HRGC/ECD | No data<br>7.1–57 ppb | No data<br>No data | Cooper et al. 1986 |
| Water (1,3-DNB, 1,3,5-TNB) | Addition of internal standard; extraction with $CH_2Cl_2$; or collection on Amberlite XAD-2, XAD-4, or XAD-8; column and dried; extraction with $CH_2C_{l2}$; extract dried over anhydrous $Na_2SO_4$; concentration and add redissolution in methanol | HRGC/ECD<br>HRGC/TEA<br>HRGC/MS | No data<br>No data<br>No data | No data<br>No data<br>No data | Feltes et al. 1990 |
| Water (1,3,5-TNB) | Extraction with toluene containing internal standard | GC/NPD | 10 µg/L | 83–102 | Army 1986 |

**TABLE 6-2. Analytical Methods for Determining 1,3-DNB and 1,3,5-TNB in Environmental Samples (continued)**

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Water (1,3,5-TNB) | Filtration; injection of sample directly or dilution and injection; elution from reverse-phase HPLC column with methanol/water | HPLC/UV | No data | 94–102 | Army 1986 |
| Tap water (1,3-DNB and 1,3,5-TNB) | Collection on Amberlite XAD® resin; elution with ethyl acetate | HRGC/ECD | <0.1 µg/L | 80–88 (1,3-DNB); 74–89 (1,3,5-TNB) | Richard and Junk 1986 |
| Tap water, waste water effluents (NB) | Extraction with Freon-TF; concentration | GC/FID | 0.12 µg/L | 97–100 | Austern et al. 1975 |
| Waste water effluents (1,3-DNB) | Addition of internal standard; elution from Sep-Pak $C_{18}$ reverse-phase column with methanol/water | HPLC/UV | ~0.2 mg/L | No data | Army 1983 |
| Surface water (1,3,5-TNB) | Collection on Amberlite XAD-2/4/8; dry; desorption with $CH_2Cl_2$ ; dry on anhydrous $Na_2SO_4$; solvent exchange to methanol; concentration; elution from reverse-phase column with methanol/water | HPLC/UV | 50 ng/L | 85–105 | Feltes and Levsen 1989 |
| Groundwater (1,3-DNB and 1,3,5-TNB) | Collection on Hayesep R solid sorbent cartridge; elution with acetone; concentration; addition of internal standard; dilution with methanol/water; elution from HPLC column with methanol/water | HPLC/UV/UV/PC | No data | No data | Army 1989 |
| Groundwater, soil/sediment, solid waste (NB) | Sample extraction and clean-up methods recommended by EPA for the specific matrix | GC/MS | 1.9 µg/L | 35–114 (water); 23–120 (soil) | EPA 1986a |

**TABLE 6-2. Analytical Methods for Determining 1,3-DNB and 1,3,5-TNB in Environmental Samples (continued)**

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Soil (1,3-TNB) | Sample dried, sieved and ground; extraction with ultrasonication in acetonitrile containing internal standard; dilution with aqueous $CaCl_2$ ; filtration; elution from reverse-phase column with methanol/water | HPLC/UV | 0.11 µg/g (1,3-DNB); 0.29 µg/g (1,3,5-TNB) | 105 (1,3-DNB); 119 (1,3,5-TNB) | Bauer et al. 1990; Jenkins et al. 1989 |
| Wine, beer, cider (NB) | Direct measurement | DPV CV | 0.5 mg/L | No data | Lorenzo et al. 1988 |

$CaCl_2$ = Calcium chloride; $CH_2Cl_2$ = Dichloromethane (methylene chloride); $CCl_4$ = Carbon tetrachloride; CV = Cyclic voltammetry; DPV = Differential pulse voltammetry; ECD = Electron capture detection; EPA = Environmental Protection Agency; FID = Flame ionization detection; GC = Gas chromatography;HCl = Hydrochloric acid; HPLC = High-performance liquid chromatography; HRGC = High-resolution gas chromatography; MS = Mass spectrometry; $N_2$ = Nitrogen gas; $Na_2SO_4$ = Sodium sulfate; NB = Nitrobenzene; NPD = Nitrogen-phosphorus detection; PC = Photoconductivity; TEA = Thermal energy analyzer; UV = Ultraviolet detector

sub to a mid-ppb range depending on the method, contamination level of the sample, efficiency of the extraction procedure, selectivity of the method, specific analyte (nitrobenzene, 1,3-DNB, or 1,3,5-TNB), and other method variables. The sensitivity of the GC-based methods is in the low-ppb range, with the limited data suggesting that ECD and FID may be slightly more sensitive than the other tested detectors (Austem et al. 1975; Richard and Junk 1986). However, TEA (Feltes et al. 1990), nitrogen-phosphorus (Army 1986), and MS (EPA 1986a; Feltes et al. 1990; Stemmler and Hites 1987) detectors are considered much more selective. GC/MS using a low-resolution instrument in electron ionization mode is the method recommended by EPA (EPA 1986a) because of its selectivity and sensitivity. However, other studies have shown that negative chemical ionization (NCI) and electron capture NCI are more sensitive and reliable than the EPA method (Feltes et al. 1990; Stemmler and Hites 1987). HPLC provides an alternative to GC-based methods (Army 1983, 1989; Feltes and Levsen 1989). The HPLC methods available are sensitive (detection limits ranging from low ppt to low ppb), relatively selective, reproducible, and reliable, in that they maintain the integrity of samples (sample decomposition may occur in heated zones of GC injectors). HPLC is also simple and rapid, requiring little sample preparation. A modification of the HPLC/UV method couples two UV detectors and a PC detector (Army 1989). This arrangement of detectors can improve the selectivity of HPLC substantially. An MS technique that allows direct injection of a water sample has also been tested (Yinon and Laschever 1982). The detection limit was only in the low-ppm range, but its selectivity makes it a good method for screening samples for further analysis. Assays based on COD and TOC (Roth and Murphy 1978) are well-established standard methods for determining organic pollution in water, but they are not selective for nitrobenzene compounds.

HPLC/UV was the only method located for measuring nitrobenzene, 1,3-DNB, and 1,3,5-TNB in soil (Army 1985a; Bauer et al. 1990; Jenkins and Grant 1987; Jenkins et al. 1989). This method, developed by the Army, has been extensively tested and has been proven to be selective and reliable, giving high recoveries and good precision for complex samples. Sensitivity in the low-ppm range has been reported. A similar method, with less rigid sample clean-up, had recoveries for nitrobenzene that varied widely (Grob and Cao 1990). This shows the importance of sample extraction and clean-up with regard to results when the matrix is complex.

Cyclic voltammetry and differential pulse voltammetry have been used to analyze wine, beer, and cider for nitrobenzene (Lorenzo et al. 1988). While no detection limits were reported, amounts as low as 0.5 mg/L were easily detected and precision was excellent (±5% CV). An advantage of this method is

that the analyte can be measured by direct insertion of the electrode in the solution. The method should also apply to the detection of 1,3-DNB and 1,3,5-TNB in solutions because it is based on polarographic determination of the nitro group. However, it is not as selective as HPLC- and GC-based methods. MS/MS has been investigated as a screening method for explosives (McLuckey et al. 1985), but no data on the sensitivity and reliability of this method were available. A supercritical fluid capillary chromatographic method with FID detection has been proposed for the determination of a broad range of compounds (including nitroaromatics) in solid wastes (Pospisil et al. 1991). The method was used to chromatograph over 270 compounds on a single column within 1 hour.

## 6.3 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 1,3-DNB and 1,3,5-TNB is available. Where adequate information is not available, ATSDR, in conjunction with 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 1,3-DNB and 1,3,5-TNB.

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 or eliminate the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

### 6.3.1 dentification of Data Needs

**Methods for Determining Biomarkers of Exposure and Effect.** Very few methods were located that could be used to determine exposure to 1,3-DNB or 1,3,5-TNB in humans. A spectrophotometric method exists (Dangwal and Jethani 1980) but is selective for nitro and amino benzene-based compounds, not for 1,3-DNB and 1,3,5-TNB specifically. The best methods for determination of exposure to 1,3-DNB and 1,3,5-TNB are HRGUECD (Bailey et al. 1988) and GC/MS (McEuen and Miller 1991). To date, only HRGUECD has been used quantitatively, and only

for detection of 1,3-DNB and metabolites in blood and urine. The method has a detection limit of 10 ppb, a recovery of 110% and a coefficient of variation of 23% (Bailey et al. 1988). Since there is no database for the levels of 1,3-DNB and 1,3,5-TNB in human biological tissues or body fluids (other than in cases of accidental exposure), it is not possible to determine whether the existing analytical methods are sensitive enough to measure the background levels of the parent compound or metabolites in the general population or to measure the concentration levels at which biological effects occur in humans. Further testing and improvement of existing methods and development of new methods are needed for monitoring populations with potential for exposure to 1,3-DNB or 1,3,5-TNB.

Methemoglobinemia is a primary biomarker of effect for 1,3-DNB and 1,3,5-TNB. Well-established and reliable methods exist for monitoring methemoglobin formation using a complete blood count (Ishihara et a1.1976). However, methemoglobinemia is not a specific effect of 1,3-DNB and 1,3,5-TNB; other chemicals also cause methemoglobin formation. Other effects of exposure to 1,3-DNB and 1,3,5-TNB (cyanosis, headache, nausea, dizziness) are very general and cannot be quantified. Therefore, it would be useful to conduct further research to develop biomarkers of effect of exposure to 1,3-DNB and 1,3,5-TNB.

**Methods for Determining Parent Compounds and Degradation Products in Environmental Media.** Several methods for determining 1,3-DNB and 1,3,5-TNB in environmental media have been developed and tested. In addition, some methods that have been developed for detection of nitrobenzene can also be used for detection of 1,3-DNB and 1,3,5-TNB (Cooper et al. 1986; Dangwal 1981; Kebbekus and Bozzelli 1982; Lorenzo et al. 1988). The methods expected to be most sensitive and selective for detecting the analytes in air are GC-based with detection by FID or NPD (Andersson et al. 1983; Cooper et al. 1986; Kebbekus and Bozzelli 1982). More data on these methods when used specifically to test for 1,3-DNB and 1,3,5-TNB are needed, because Andersson et al. (1983) provided no information regarding the detection limit of the GUFID method used for the determination of 1,3-DNB in air, and very few published methods are available that give that information. Both HPLC/UV and GC (high-resolution or low-resolution), combined with one of several detectors (NPD, ECD, FID, TEA, and MS), yield good results when water is analyzed for nitrobenzene compounds (Army 1983, 1986, 1989; Austem et al. 1975; EPA 1986a; Feltes and Levsen 1989; Feltes et al. 1990; Richard and Junk 1986; Stemmler and Hites 1987). Some of these methods have not been fully developed for analysis of 1,3-DNB and 1,3,5-TNB (Army 1983, 1989; Austem et al. 1975; EPA 1986a; Stemmler and Hites 1987). A detection limit of <0.1 μg/L and a

recovery of 74-89s has been reported for 1,3-DNB and 1,3,5-TNB in tap water by HRGC/ECD methodology (Richard and Junk 1986). Further testing of these methods specifically designed to determine their usefulness for measuring 1,3-DNB and 1,3,5-TNB in water would be helpful because no detection limit has been reported for the determination of these compounds in water by other promising methods, such as HPLC/UV/UV/PC (Army 1989) and GC/NPD following sample concentration by a suitable solid adsorbent during collection. The method currently used for detection of 1,3-DNB and 1,3,5-TNB in soil is reliable (Army 1985a; Jenkins et al. 1989), but increased sensitivity would allow better detection of trace levels in potentially contaminated soils. Since the background levels of 1,3-DNB and 1,3,5-TNB in ambient air, water and soil have not been established, it is not possible to determine whether the existing methods would be sensitive enough to measure 1,3-DNB and 1,3,5-TNB concentrations in background samples. Very little data were located on methods of analyzing for 1,3-DNB and 1,3,5-TNB in other media. The methods located for detection of the analytes in both beverages (Lorenzo et al. 1988) and explosives (McLuckey et al. 1985) were still in the developmental stages. Methods for analyzing these compounds in other media, especially in foods, plants, and aquatic and terrestrial organisms, are needed.

Some of the aerobic and anaerobic biodegradation products of 1,3-DNB in the environment are 1,3-nitroaniline, 1,3-phenylenediamine, 1,3-nitrophenol, 1,3-aminophenol and resorcinol (1,3-dihydroxyphenol) (Dey and Godbole 1986; Hallas and Alexander 1983; Reddy et al. 1993). A reversed-phase HPLWUV method is available for the determination of reduction products of 1,3-dinitrobenzene in the presence of 1,3-DNB and 1,3,5-TNB (Reddy et al. 1993). The derivatization of the amino-group with trifluoroacetic anhydride may increase the sensitivity of 1,3-nitroaniline and 1,3-phenylenediamine determination by the HPLCXJV method (Preslan et al. 1993). However, this method has not been standardized for the determination of reduction products of 1,3-DNB and 1,3,5-TNB.

### 6.3.2 Ongoing Studies

No ongoing studies regarding analytical methods were’ located for either 1,3-DNB or 1,3,5-TNB.

# 7. REGULATIONS AND ADVISORIES

The international, national, and state regulations and guidelines regarding 1,3-DNB and 1,3,5-TNB in air, water, and other media are summarized in Table 7-l.

ATSDR has derived two MRL values for 1,3-DNB. An acute-duration oral MRL of 0.08 mg/kg/day was derived for 1,3-DNB based on a NOAEL for testicular damage in male rats administered a single dose of 1,3-DNB by gavage (Linder et al. 1990).

An intermediate-duration oral MRL of 0.0005 mg/kg/day was derived for 1,3-DNB based on a LOAEL for hematological effects in male rats administered 1,3-DNB by gavage intermittently for 12 weeks (Linder et al. 1986).

The EPA reference dose (RfD) is $1x10^{-4}$ mg/kg/day for 1,3-DNB and is based on spleen enlargement in rats (IRIS 1994). The EPA RfD for 1,3,5-TNB is $5x10^{-5}$ mg/kg/day and is extrapolated from the RfD for 1,3-DNB based on the structural similarity.

EPA has assigned 1,3-DNB a weight-of-evidence carcinogenic classification of D, which indicates that 1,3-DNB is not classifiable as to human carcinogenicity (IRIS 1994). EPA has not assigned a weightof-evidence carcinogenic classification for 1,3,5-TNB.

1,3-DNB and 1,3,5-TNB are designated as hazardous substances (EPA 1978, 1987b) and are subject to groundwater monitoring requirements (EPA 1987a, 1988b).

The transportation.of explosives, including 1,3-DNB and 1,3,5-TNB, must be in accordance with the Department of Transportation hazardous material regulations (49 CFR 171-190) and the motor carrier safety regulations (49 CFR 390-398). Numerous states have established regulations on explosives for air quality control, solid waste disposal, storage, manufacture, and use.

OSHA requires employers of workers who are occupationally exposed to 1,3-DNB to institute engineering controls and work practices to reduce and maintain employee exposure at or below permissible exposure limits (PEL). The employer must use engineering and work practice controls, if feasible, to reduce exposure to or below an 8-hour time-weighted average (TWA) of 1.0 $mg/m^3$ (skin

designation) for 1,3-DNB. No value has been designated for 1,3,5-TNB. Respirators must be provided and used during the time period necessary to install or implement feasible engineering and work practice controls. Also, special protective measures should be taken to significantly reduce or preclude skin contact.

1,3-DNB is regulated as a toxic chemical under the Emergency Planning and Community Right-tol$ now Act (EPCRA) (EPA 1986c) and the Toxic Substances Control Act (TSCA) for preliminary assessment of new products (EPA 1982a) and health and safety data reporting (EPA 1982b).

The Resource Conservation and Recovery Act (RCRA) identifies both compounds for groundwater monitoring at hazardous waste management facilities (EPA 1987a). RCRA also designates 1,3,5-TNB as a hazardous waste when it occurs as a discarded commercial product, off-spec species, container - residue, or spill residue (EPA 1980a).

# Table 7-1. Regulations and Guidelines Applicable to 1,3-DNB and 1,3,5-TNB

| Agency | Description | Information | Reference |
|---|---|---|---|
| NATIONAL | | | |
| Regulations: | | | |
| a. Air: | | | |
| OSHA | PEL TWA (skin designation) (1,3-DNB) | 1 $mg/m^3$ | OSHA 1989a (29 CFR 1910.1000) OSHA 1989b |
| OSHA | Meets criteria for medical records (1,3-DNB) | Yes | OSHA 1987 (29 CFR 1910.20) OSHA 1988 |
| b. Other | | | |
| EPA-OERR | Reportable quantity: | | |
| | 1,3-DNB | 100 lb. | EPA 1985a (40 CFR 302.4) EPA 1985b |
| | 1,3,5-TNB | 10 lb. | |
| EPA-OERR/CEPP | Chemicals and chemical categories to which this part applies (1,3-DNB) | 25,000 lbs. mfd. or processed; 10,000 lbs. otherwise used | EPA 1988c (40 CFR 372.65) |
| EPA-OSW | Designated as a hazardous substance (1,3-DNB, 1,3,5-TNB) | Yes | EPA 1978 (40 CFR 116.4) EPA 1987b |
| | Listing as a hazardous waste: Discarded commercial chemical product off-specification species, container residues, and spill residues thereof (1,3,5-TNB) | Yes | EPA 1980a (40 CFR 261.33) EPA 1980b |
| | Municipal Solid Waste Landfills: Appendix II | | EPA 1991a (40 CFR 258) |
| | 1,3-DNB | 20 µg/L | |
| | TNB (sym) | 10 µg/L (Practical Quantitation Limit) | |
| | Groundwater Monitoring List: Appendix IX | | EPA 1987a (40 CFR 264) |
| | 1,3-DNB | 10 µg/L | |
| | 1,3,5-TNB | 10 µg/L (Practical Quantitation Limit) | |
| | Hazardous Constituents: Appendix VIII (1,3,5-TNB) | none | EPA 1988a (40 CFR 261) |
| | Treatment Standards Expressed as Specified Technologies (1,3,5-TNB) | none | EPA 1986b (40 CFR 268.42) |
| EPA/OPTS | Chemical lists and reporting periods (1,3-DNB) | none | EPA 1982a (40 CFR 712.30) |
| | Substances and listed mixtures to which this subpart applies (1,3-DNB) | none | EPA 1988d (40 CFR 716.120) |

## Table 7-1. Regulations and Guidelines Applicable to 1,3-DNB and 1,3,5-TNB (continued)

| Agency | Description | Information | Reference |
|---|---|---|---|
| NATIONAL (Cont.) | | | |
| DOT | Poison B (1,3,5-DNB/solid and solution) | Yes | DOT 1989a (49 CFR 172.101) DOT 1989b |
| | Class A explosive (high explosive); Domestic transportation limited to road and water (cargo only, in magazines) (1,3,5-TNB/dry) | Yes | |
| DOT | Designated as a hazardous substance subject to requirements for packaging, labeling, and transportation (1,3-DNB, 1,3,5-TNB) | Yes | DOT 1989a (49 CFR 172.101) Appendix A DOT 1989b |
| Guidelines: | | | |
| a. Air | | | |
| ACGIH | TLV TWA (skin designation) (1,3-DNB) | 1.0 mg/m$^3$ 0.15 ppm | ACGIH 1994 |
| NIOSH | Recommended Exposure Limit (10-hour TWA) (DNB) | 1.0 mg/m$^3$ | NIOSH 1992 |
| b. Water | | | |
| EPA/OW | 10-d Health Advisory (child) | 0.04 mg/L | EPA 1994 |
| | 1-d Health Advisory (child) | 0.04 mg/L | EPA 1994 |
| | Lifetime Health Advisory (adult) | 0.001 mg/L | EPA 1994 |
| EPA/ODW | Drinking Water Guideline | 2.0 µg/L | FSTRAC 1990 |
| c. Other | | | |
| EPA | 1,3-DNB: | | IRIS 1994 |
| | RfD (oral) | 1.0x10$^{-4}$ mg/kg/day | |
| | Carcinogenic classification | D[a] | |
| | Unit risk (air) | ND | |
| | Unit risk (water) | ND | |
| | 1,3,5-TNB: | | IRIS 1994 |
| | Rfd (oral) | 5.0x10$^{-5}$mg/kg/day | |
| | Carcinogen classification | ND | |
| | Unit risk (air) | ND | |
| | Unit risk (water) | ND | |
| STATE | | | |
| Regulations and Guidelines: | | | |
| a. Air: | | | |
| | Acceptable Ambient Air Concentrations (1,3-DNB) | | NATICH 1992 |
| CT | 8 hr avg. time | 20.0 µg/m$^3$ | |

## Table 7-1. Regulations and Guidelines Applicable to 1,3-DNB and 1,3,5-TNB (continued)

| Agency | Description | Information | Reference |
|---|---|---|---|
| **STATE** (cont.) | | | |
| FL-Tampa | 8 hr avg. time | 0.01 $mg/m^3$ | |
| FL-Ft. Lauderdale | 8 hr avg. time | 0.01 $mg/m^3$ | |
| MD | | 0.00 | |
| ME | | 0.00 | |
| ND | 8 hr avg. time | 0.01 $mg/m^3$ | |
| NV | 8 hr avg. time | 0.024 $mg/m^3$ | |
| NY | 1 yr avg. time | 3.30 | |
| SC | 24 hr avg. time | 10.0 $\mu g/m^3$ | |
| TX | 30 min avg. time | 10.0 $\mu g/m^3$ | |
| TX[9] | 30 min. avg. time | 1.00 $\mu g/m^3$ | |
| VA | 24 hr avg. time | 17.0 $\mu g/m^3$ | |
| KY | Significant emission levels of toxic air pollutants (1,3-DNB) | $2.551 \times 10^{-4}$ pounds/hour | NREPC 1986 (410 401 63:022) |
| WI | Hazardous air contaminants with acceptable ambient air concentrations (1,3-DNB) | Yes | WAC 1988 |
| b. Other | Transportation of explosives is in accordance with the U.S. Department of Transportation hazardous materials regulations 49 CFR 171-190 and the motor carrier safety regulations 49 CFR 390-398 with some exceptions or additional requirements that vary from state to state. (1,2-DNB; 1,3,5-TNB). | | CELDs 1991 |
| AL | | Yes | |
| AK | | Yes | |
| AZ | | Yes | |
| CA | | Yes | |
| CT | | Yes | |
| CO | | Yes | |
| DE | | Yes | |
| FL | | Yes | |
| GA | | Yes | |
| HI | | Yes | |
| ID | | Yes | |

## Table 7-1. Regulations and Guidelines Applicable to 1,3-DNB and 1,3,5-TNB (continued)

| Agency | Description | Information | Reference |
|---|---|---|---|
| **STATE** (cont.) | | | |
| IN | | Yes | |
| IA | | Yes | |
| LA | | Yes | |
| MD | | Yes | |
| MA | | Yes | |
| MI | | Yes | |
| MN | | Yes | |
| MS | | Yes | |
| MO | | Yes | |
| MT | | Yes | |
| NE | | Yes | |
| NJ | | Yes | |
| NM | | Yes | |
| NC | | Yes | |
| ND | | Yes | |
| NY | | Yes | |
| OH | | Yes | |
| OR | | Yes | |
| RI | | Yes | |
| SD | | Yes | |
| TN | | Yes | |
| TX | | Yes | |
| UT | | Yes | |
| VA | | Yes | |
| VT | | Yes | |
| WA | | Yes | |
| WA-DC | | Yes | |
| WV | | Yes | |
| WI | | Yes | |
| WY | | Yes | |

## Table 7-1. Regulations and Guidelines Applicable to 1,3-DNB and 1,3,5-TNB (continued)

| Agency | Description | Information | Reference |
|---|---|---|---|
| **STATE** (cont.) | | | |
| | Rules and regulations for air quality control and/or solid waste disposal have been established for explosives in general. The regulations vary from state to state (1,3-DNB; 1,3,5-TNB). | | CELDs 1991 |
| AL | Pretreatment standards for discharge | Yes | |
| | Hazardous waste: Thermal treatment | Yes | |
| AZ | Solid waste collection | Yes | |
| AR | Solid waste storage and collection | Yes | |
| CO | Fugitive dust | Yes | |
| CT | Hazardous waste: Thermal treatment | Yes | |
| FL | Hazardous waste: Thermal treatment | Yes | |
| GA | Open burning | Yes | |
| IL | Sewer discharge | Yes | |
| IN | Open burning | Yes | |
| KY | Hazardous waste management | Yes | |
| LA | Open burning | Yes | |
| MN | Hazardous waste management | Yes | |
| NH | Open burning | Yes | |
| NJ | Hazardous waste management | Yes | |
| NM | Hazardous waste management | Yes | |
| NV | Hazardous waste management | Yes | |
| NC | Hazardous waste: Thermal treatment | Yes | |
| ND | Fugitive emissions | Yes | |
| PA | Fugitive emissions | Yes | |
| SC | Open burning | Yes | |
| TN | Hazardous waste: Thermal treatment | Yes | |
| UT | Hazardous waste management | Yes | |
| VT | Open burning | Yes | |
| VA | Solid waste management | Yes | |
| WI | Open burning and malodorous emissions | Yes | |
| | Hazardous Constituent | | CELDs 1993 |
| CO | (1,3-DNB; 1,3,5-TNB) | none | |
| IL | (1,3-DNB; 1,3,5-TNB) | none | |

## Table 7-1. Regulations and Guidelines Applicable to 1,3-DNB and 1,3,5-TNB (continued)

| Agency | Description | Information | Reference |
|---|---|---|---|
| STATE (cont.) | | | |
| LA | (1,3-DNB; 1,3,5-TNB) | none | |
| MN | (1,3-DNB; 1,3,5-TNB) | none | |
| ND | (1,3-DNB; 1,3,5-TNB) | none | |
| WV | (1,3-DNB; 1,3,5-TNB) | none | |
| WI | (1,3-DNB; 1,3,5-TNB) | none | |
| | Groundwater Monitoring | | CELDs 1993 |
| CO | (1,3-DNB; 1,3,5-TNB) | none | |
| IL | (1,3-DNB; 1,3,5-TNB) | none | |
| LA | (1,3-DNB; 1,3,5-TNB) | none | |
| MN | (1,3-DNB; 1,3,5-TNB) | none | |
| WV | (1,3-DNB; 1,3,5-TNB) | none | |
| WI | (1,3-DNB; 1,3,5-TNB) | none | |
| | Explosive control laws regulate storage, manufacture, and use. The regulations vary from state to state (1,3-DNB; 1,3,5-TNB). | | CELDs 1991 |
| AK | | Yes | |
| CA | | Yes | |
| CT | | Yes | |
| GA | | Yes | |
| HI | | Yes | |
| IN | | Yes | |
| IA | | Yes | |
| KS | | Yes | |
| NJ | | Yes | |
| MS | | Yes | |
| NB | | Yes | |
| NJ | | Yes | |
| OK | | Yes | |
| OR | | Yes | |
| WA-DC | | Yes | |
| WV | | Yes | |

## Table 7-1. Regulations and Guidelines Applicable to 1,3-DNB and 1,3,5-TNB (continued)

| Agency | Description | Information | Reference |
|---|---|---|---|
| **STATE** (cont.) | | | |
| WI | | Yes | |
| KY | Listing as a hazardous waste (1,3,5-TNB) | Yes | NREPC 1986 (401 MASS 31:040) |

NOTE: Units in table reflect values and units of measure designated by each agency in its regulations or advisories.

[a]Not classifiable as to human carcinogenicity.

ACGIH = American Conference of Governmental and Industrial Hygienists; CELDs = Comprehensive Environmental Legislative Database; CEPP = Chemical Emergency Preparedness and Prevention; CFR = Code of Federal Regulations; D = not classifiable as to human carcinogenicity; DOT = Department of Transportation; EPA = Environmental Protection Agency; FSTRAC = Federal-State Toxicology and Regulatory Alliance Committee; IRIS = Integrated Risk Information System; ND = No data; NIOSH = National Institute for Occupational Safety and Health; ODW = Office of Drinking Water; OERR = Office of Emergency and Remedial Response; OPTS = Office of Pesticides and Toxic Substances; OSHA = Occupational Safety and Health Administration; OSW = Office of Solid Waste; OW = Office of Water; PEL = Permissible exposure limit; REL = Recommended Exposure Limits; RfD = Reference dose; TLV = Threshold limit value; TWA = Time weighted average

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# 9. GLOSSARY

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

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

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

**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.

**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.

**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.

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

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

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

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

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

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

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

***In vivo*** -- Occurring within the living organism.

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

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

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

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

**Lethal Time($_{50}$) ($LT_{50}$)** -- 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.

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

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

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

**Neurotoxicity** -- The occurrence of adverse effects on the nervous system following exposure to 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 Coeficient ($K_{OW}$)** -- The equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution.

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

**$q_1$*** -- The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The $q_1$* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually μg/L for water, mg/kg/day for food, and μg/m3 for air).

**Reference Dose (RfD)** -- An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the NOAEL (from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional. modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

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

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

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

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

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

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

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

**Uncertainty Factor (UF) --** A factor used in operationally deriving the RfD from experimental data. Ufs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of 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 LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

# APPENDIX A

# USER'S GUIDE

## Chapter 1

### Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

## Chapter 2

### Tables and Figures for Levels of Significant Exposure (LSE)

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

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 2-1 and Figure 2-1 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 2-1**

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

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

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

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

(5) **Species** The test species, whether animal or human, are identified in this column, Section 2.4, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 2.3, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) **Exposure Frequency/Duration** The duration of the study and the weekly and daily exposure regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to toxaphene 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 dernWocu1a.r. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, 1 systemic effect (respiratory) was investigated.

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

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

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

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

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

**LEGEND**

**See Figure 2-1**

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

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

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

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

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

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

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

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

SAMPLE

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

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

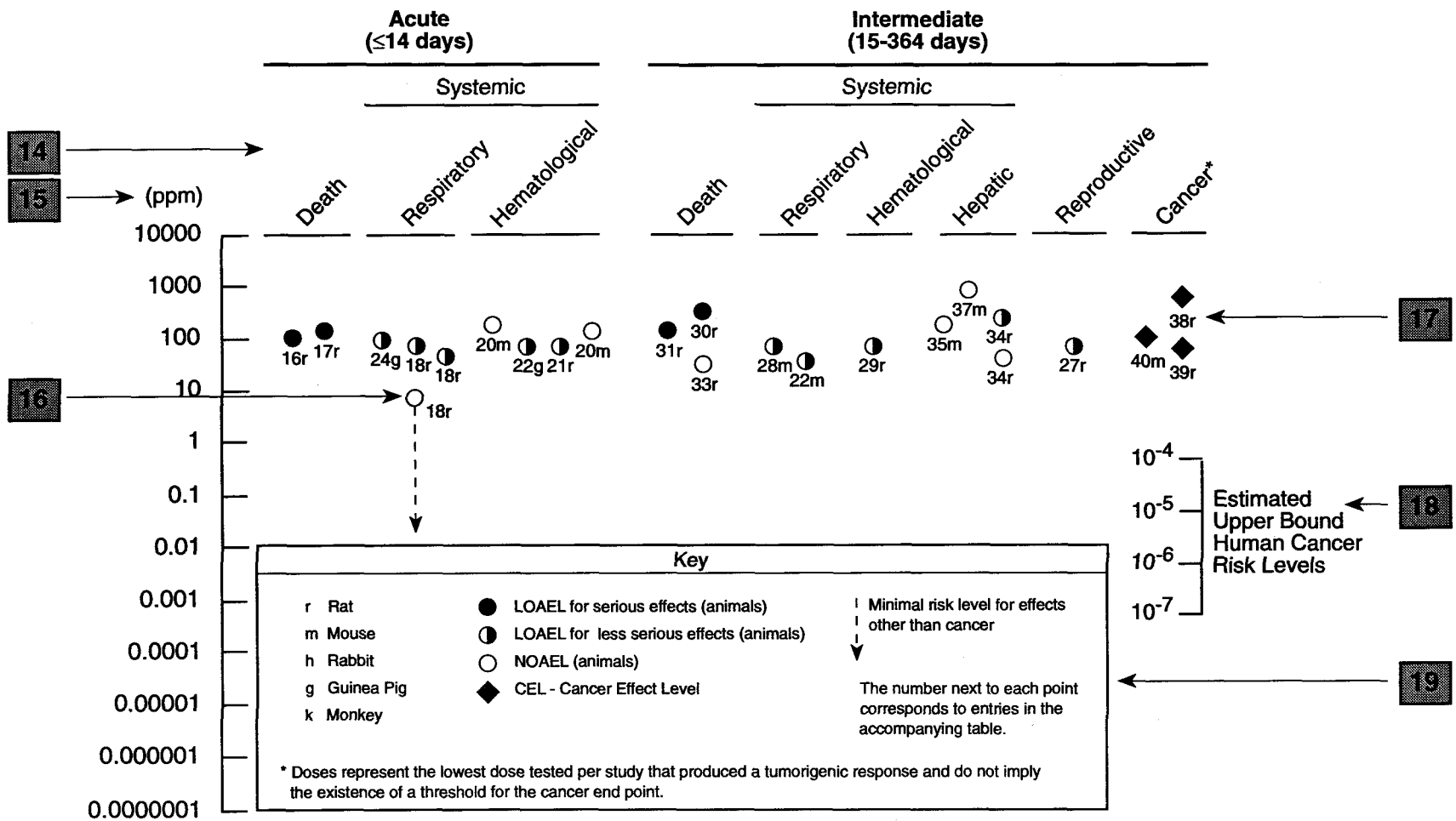
[a] The number corresponds to entries in Figure 2-1.

12 → [b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5 x $10^{-3}$ 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).

CEL = cancer effect level; d = days(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

SAMPLE

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



## Chapter 2 (Section 2.4)

### Relevance to Public Health

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

What effects are known to occur in humans?

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

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

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

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal risk levels (MRLs) for noncancer endpoints (if derived) and the endpoints from which they were derived are indicated and discussed.
Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Data Needs section.

### Interpretation of Minimal Risk Levels

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

MRL users should be familiar with the toxicologic information on which the number is based, Chapter 2.4, “Relevance to Public Health,” contains basic information known about the substance. Other sections such as 2.6, “Interactions with Other Substances,” and 2.7, “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 (RIDS).

**APPENDIX A**

To derive an MRL, ATSDR generally selects the most sensitive endpoint 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 endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

# APPENDIX B

# ACRONYMS, ABBREVIATIONS, AND SYMBOLS

| | |
|---|---|
| ACGIH | American Conference of Governmental Industrial Hygienists |
| ADME | Absorption, Distribution, Metabolism, and Excretion |
| atm | atmosphere |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| BCF | bioconcentration factor |
| BSC | Board of Scientific Counselors |
| C | Centigrade |
| CDC | Centers for Disease Control |
| CEL | Cancer Effect Level |
| CERCLA | Comprehensive Environmental Response, Compensation, and Liability Act |
| CFR | Code of Federal Regulations |
| CLP | Contract Laboratory Program |
| cm | centimeter |
| CNS | central nervous system |
| d | day |
| DHEW | Department of Health, Education, and Welfare |
| DHHS | Department of Health and Human Services |
| DOL | Department of Labor |
| ECG | electrocardiogram |
| EEG | electroencephalogram |
| EPA | Environmental Protection Agency |
| EKG | see ECG |
| F | Fahrenheit |
| $F_1$ | first filial generation |
| FAO | Food and Agricultural Organization of the United Nations |
| FEMA | Federal Emergency Management Agency |
| FIFRA | Federal Insecticide, Fungicide, and Rodenticide Act |
| fpm | feet per minute |
| ft | foot |
| FR | Federal Register |
| g | gram |
| GC | gas chromatography |
| gen | generation |
| HPLC | high-performance liquid chromatography |
| hr | hour |
| IDLH | Immediately Dangerous to Life and Health |
| IARC | International Agency for Research on Cancer |
| ILO | International Labor Organization |
| in | inch |
| Kd | adsorption ratio |
| kg | kilogram |
| kkg | metric ton |
| $K_{oc}$ | organic carbon partition coefficient |
| $K_{ow}$ | octanol-water partition coefficient |
| L | liter |
| LC | liquid chromatography |

APPENDIX B

| | |
|---|---|
| $LC_{Lo}$ | lethal concentration, low |
| $LC_{50}$ | lethal concentration, 50% kill |
| $LD_{Lo}$ | lethal dose, low |
| $LD_{50}$ | lethal dose, 50% kill |
| LOAEL | lowest-observed-adverse-effect level |
| LSE | Levels of Significant Exposure |
| m | meter |
| mg | milligram |
| min | minute |
| mL | milliliter |
| mm | millimeter |
| mm Hg | millimeters of mercury |
| mmol | millimole |
| mo | month |
| mppcf | millions of particles per cubic foot |
| MRL | Minimal Risk Level |
| MS | mass spectrometry |
| NIEHS | National Institute of Environmental Health Sciences |
| NIOSH | National Institute for Occupational Safety and Health |
| NIOSHTIC | NIOSH's Computerized Information Retrieval System |
| ng | nanogram |
| 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 |
| NPL | National Priorities List |
| NRC | National Research Council |
| NTIS | National Technical Information Service |
| NTP | National Toxicology Program |
| OSHA | Occupational Safety and Health Administration |
| PEL | permissible exposure limit |
| pg | picogram |
| pmol | picomole |
| PHS | Public Health Service |
| PMR | proportionate mortality ratio |
| ppb | parts per billion |
| ppm | parts per million |
| ppt | parts per trillion |
| REL | recommended exposure limit |
| RfD | Reference Dose |
| RTECS | Registry of Toxic Effects of Chemical Substances |
| sec | second |
| SCE | sister chromatid exchange |
| SIC | Standard Industrial Classification |
| SMR | standard mortality ratio |
| STEL | short term exposure limit |
| STORET | STORAGE and RETRIEVAL |

APPENDIX B

| | |
|---|---|
| TLV | threshold limit value |
| TSCA | Toxic Substances Control Act |
| TRI | Toxics Release Inventory |
| TWA | time-weighted average |
| U.S. | United States |
| UF | uncertainty factor |
| 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 |
| δ | delta |
| γ | gamma |
| μm | micron |
| μg | microgram |

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