TOXICOLOGICAL PROFILE FOR 2,4,6-TRINITROTOLUENE

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.
Administrator
Agency for Toxic Substances and
Disease Registry

CONTRIBUTORS

CHEMICAL MANAGER(S)/AUTHORS(S):

Patricia Richter-Torres, Ph.D.
ATSDR, Division of Toxicology, Atlanta, GA

Alfred Dorsey, D.V.M.
ATSDR, Division of Toxicology, Atlanta, GA

Colette S. Hodes, Ph.D.
Sciences International, Inc., Alexandria, VA

THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Green Border Review. Green Border review assures the 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 endpoints.

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 2,4,6-trinitrotoluene. The panel consisted of the following members:

1. Dr. Lawrence Martin Holland, Private Consultant, Los Alamos, New Mexico

2. Dr. Thomas McKone, Engineer, Environmental Science Division, Lawrence Livermore National Laboratory, Livermore, California

3. Dr. Ronald Spanggord, Director Bio-Analytical Chemistry, Associate Director, Pharmaceutical Analysis Department, SRI International, Menlo Park, California

4. Dr. William George, Professor, Department of Pharmacology, Tulane University, New Orleans, Louisiana

These experts collectively have knowledge of 2,4,6-trinitrotoluene'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 2,4,6-trinitrotoluene and to emphasize the human health effects that may result from exposure to it. The Environmental Protection Agency (EPA) has identified 1,397 sites on its National Priorities List (NPL). 2,4,6-Trinitrotoluene has been found in at least 20 of these sites. However, we do not know how many of the 1,397 NPL sites have been evaluated for 2,4,6-trinitrotoluene. As EPA evaluates more sites, the number of sites at which 2,4,6-trinitrotoluene is found may change. This information is important for you to know because 2,4,6-trinitrotoluene may cause harmful health effects and because these sites are potential or actual sources of human exposure to 2,4,6-trinitrotoluene.

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 2,4,6-trinitrotoluene, 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, life-style, and state of health.

1. PUBLIC HEALTH STATEMENT

1.1 WHAT IS 2,4,6-TRINITROTOLUENE?

2,4,6-Trinitrotoluene is a yellow, odorless, solid manufactured compound that does not occur naturally in the environment. It is made by combining toluene with a mixture of nitric acid and sulfuric acid. 2,4,6-Trinitrotoluene is also known by other names such as symtrinitrotoluene, TNT, and 1 -methyl-2,4,6-trinitrobenzene. 2,4,6-Trinitrotoluene is produced in the United States only at military arsenals. It is not produced commercially. 2,4,6-Trinitrotoluene is an explosive used in military shells, bombs, and grenades, in industrial uses, and in underwater blasting. For more information on the chemical and physical properties of 2,4,6-trinitrotoluene, see Chapter 3. For more information on its production and use, see Chapter 4.

1.2 WHAT HAPPENS TO 2,4,6-TRINITROTOLUENE WHEN IT ENTERS THE ENVIRONMENT?

2,4,6-Trinitrotoluene enters the environment in waste waters and solid wastes resulting from the manufacture of the compound, the processing and destruction of bombs and grenades, and the recycling of explosives. The compound moves in surface water and through soils to groundwater. In surface water, 2,4,6-trinitrotoluene is rapidly broken down into other chemical compounds by sunlight. Microorganisms in water and sediment break down the compound more slowly. Small amounts of 2,4,6-trinitrotoluene can accumulate in fish and plants. For more information on what happens to 2,4,6-trinitrotoluene when it enters the environment, see Chapter 5.

1.3 HOW MIGHT I BE EXPOSED TO 2,4,6-TRINITROTOLUENE?

You may be exposed to 2,4,6-trinitrotoluene as a result of its movement from chemical waste disposal sites to drinking water. Children may also be exposed through eating contaminated soil. Most exposure would result from drinking contaminated water, breathing contaminated

air, or eating contaminated foods such as fruits and vegetables. 2,4,6-Trinitrotoluene has been measured at waste disposal sites in groundwater at 0.32 parts of 2,4,6-trinitrotoluene per million parts of water (ppm) and in soil at up to 13,000 ppm. We have no data on levels in air or foods. 2,4,6-Trinitrotoluene can be taken up by plants from contaminated soil and is probably present in the air as a result of disposal by burning at military sites. Therefore, intake of air and homegrown fruits and vegetables by people living near military sites may also be sources of exposure to 2,4,6-trinitrotoluene.

Worker exposure to 2,4,6-trinitrotoluene is possible as a result of its use in the production of bombs and grenades. Most workplace exposure results from breathing in 2,4,6-trinitrotoluene dust or vapor and contact with dust on the skin. For additional information on how you can be exposed to 2,4,6-trinitrotoluene, see Chapter 5.

1.4 HOW CAN 2,4,6-TRINITROTOLUENE ENTER AND LEAVE MY BODY?

2,4,6-Trinitrotoluene rapidly and completely enters your body when you breathe in air or drink water that is contaminated with this chemical. We have no information on how much 2,4,6-trinitrotoluene enters your body when it gets on your skin. We do know that it enters your body more slowly through the skin than when it is taken into your mouth.
2,4,6-Trinitrotoluene in your blood travels throughout your body to all of your organs. When 2,4,6-trinitrotoluene reaches your liver, it breaks down and changes into several different substances. Not all of these substances have been identified, and we do not know whether they are harmful or not. Most of these substances travel in your blood until they reach your kidneys and then leave your body in your urine. Studies in animals show that almost all of the 2,4,6-trinitrotoluene that enters the body breaks down and leaves the body in the urine within 24 hours. Chapter 2 contains more information on how 2,4,6-trinitrotoluene enters and leaves your body.

1.5 HOW CAN 2,4,6-TRINITROTOLUENE AFFECT MY HEALTH?

Workers involved in the production of high explosives experienced many harmful health effects as a result of exposure to 2,4,6-trinitrotoluene at their jobs. These effects included disorders of the blood, such as anemia, and abnormal liver function. However, the levels of 2,4,6-trinitrotoluene in the workplace air at the time these effects were seen ranged from less than 0.01 to 1.49 milligrams of 2,4,6-trinitrotoluene per cubic meter of air (mg/m^3). Some of the concentrations measured are higher than the level currently allowed in the workplace (0.5 mg/m^3). Similar effects on the blood and the liver have been observed in animals that either breathed or were fed 2,4,6-trinitrotoluene. In addition, studies show that animals forcefed 2,4,6-trinitrotoluene for an intermediate-duration (from 15-364 days) may have enlargement of the spleen and other harmful effects on the immune system. When people have prolonged skin contact with 2,4,6-trinitrotoluene, they may develop an allergic reaction of the skin to this chemical, such as itching and irritation. In addition, long-term exposure to 2,4,6-trinitrotoluene has been associated with the development of cataracts in people.

No information is available to indicate whether 2,4,6-trinitrotoluene causes birth defects. However, studies in animals that were treated with high doses of 2,4,6-trinitrotoluene have shown that it can cause serious effects on the male reproductive system. The available information for determining whether 2,4,6-trinitrotoluene causes cancer in humans is inadequate. However, rats that ate 2,4,6-trinitrotoluene for long periods developed tumors of the urinary bladder. Based on this study with rats, EPA has classified 2,4,6-trinitrotoluene in Group C, a possible human carcinogen.

1.6 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO 2,4,6-TRINITROTOLUENE?

There are tests to determine if you have been exposed to 2,4,6-trinitrotoluene. These tests measure 2,4,6-trinitrotoluene or its breakdown products in your blood and urine and have been used to test exposed workers. Detection of the breakdown products in your urine is a clear indication that you have been exposed. The complex and expensive equipment needed to perform these tests is generally available only at specialized laboratories. Another simpler, but less specific, test of 2,4,6-trinitrotoluene exposure is a change in the color of your urine to amber or deep red. This change results from the presence of breakdown products and may indicate that you have been exposed to 2,4,6-trinitrotoluene. None of these tests can predict whether a person exposed to 2,4,6-trinitrotoluene will experience any health effects related to the exposure. For more information on tests for exposure, see Chapters 2 and 6.

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

The government has developed regulations and guidelines for 2,4,6-trinitrotoluene. These are designed to protect the public and workers exposed to 2,4,6-trinitrotoluene from potential harmful health effects of the chemical. Since 2,4,6-trinitrotoluene is explosive, flammable, and toxic, EPA has designated it as a hazardous waste. The Department of Transportation (DOT) regulates the transport of 2,4,6-trinitrotoluene because it is a hazardous material. DOT specifies that when 2,4,6-trinitrotoluene is shipped, it must be wet with at least 10% water (by weight) and it must be clearly labeled as a flammable solid (HSDB 1994).

The Occupational Safety and Health Administration (OSHA) regulates levels of hazardous materials in the workplace. The maximum allowable amount of 2,4,6-trinitrotoluene in workroom air during an 8-hour workday, 40-hour workweek, is 0.5 mg/m^3. The National Institute for Occupational Safety and Health (NIOSH) recommends that the concentration in

workroom air be limited to 0.5 mg/m^3 for up to a 10-hour workday during a 40-hour workweek (NIOSH 1992). For more information on federal regulations about 2,4,6-trinitrotoluene, see Chapter 7.

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

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 illnesses 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 2,4,6-trinitrotoluene 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 2,4,6-trinitrotoluene based on toxicological studies and epidemiological investigations.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or 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 is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant

dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of 2,4,6-trinitrotoluene are indicated in Table 2- 1 and Figure 2- 1.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for 2,4,6-trinitrotoluene. 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 noncancerous health effects only and do not consider carcinogenic effects. MRLs 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.

2. HEALTH EFFECTS

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), 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.

2,4,6-Trinitrotoluene is the most widely used military high explosive. 2,4,6-Trinitrotoluene has been used extensively in the manufacture of explosives since the beginning of this century. Its use greatly increased during World War I when its toxicity was first observed (Hathaway 1985). Based on data from a number of epidemiological studies of exposed workers, numerous adverse health effects such as anemia (reduced number of red blood cells and reduced hemoglobin and hematocrit), Iiver function abnormalities, respiratory complications, and possibly aplastic anemia have been observed at 2,4,6-trinitrotoluene exposure levels below the former standard of 1.5 mg/m^3 (Hathaway 1977). Because of the seriousness of effects caused by exposure to relatively low levels of 2,4,6-trinitrotoluene in the workplace, the threshold limit value (TLV) was lowered to 0.5 mg/m^3 (ACGIH 1993).

As more information became available, and especially after World War II, the incidence of toxic effects associated with handling of 2,4,6-trinitrotoluene decreased sharply. This decrease is primarily the result of the introduction of protective measures for ammunition workers (e.g., protective clothing, change of contaminated clothes, use of indicator soap, and mandatory bathing) and the improvement of ammunition plant ventilation systems (Army 1978a; Goodwin 1972).

2.2.1 Inhalation Exposure

All the studies presented in the section on inhalation exposure are epidemiological reports or case reports of occupational exposure. In some of the studies, inhalation exposure may have occurred simultaneously with dermal exposure. Therefore, some of the effects described in this section may be due in part to dermal exposure to 2,4,6-trinitrotoluene. Furthermore, in several studies the precise levels of exposure are not known. Consequently, results from those studies are not presented in a table or figure.

2.2.1.1 Death

Historically, the greatest number of deaths among munitions workers were caused by adverse effects of 2,4,6-trinitrotoluene on the liver. Initial clinical symptoms included nausea, vomiting, pain in the abdomen, fatigue, dizziness, petechiae, and jaundice. Exposure to 2,4,6-trinitrotoluene eventually led to 475 deaths in the United States during World War I (McConnell and Flinn 1946). It is important to note, however, that the route of exposure was probably not exclusively inhalation, but also dermal.

No studies were located regarding death in animals after inhalation exposure to 2,4,6-trinitrotoluene.

2.2.1.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, musculoskeletal, or renal effects in humans or animals after inhalation exposure to 2,4,6-trinitrotoluene.

Respiratory Effects. Extremely limited information is available regarding respiratory effects in humans after inhalation exposure to 2,4,6-trinitrotoluene. One study of occupational exposure (Morton et al. 1976) reported several cases of respiratory difficulties in ammunition plant workers who were exposed to 2,4,6-trinitrotoluene in the air at a level that was well above the current TLV of 0.5 mg/m^3 (ACGIH 1993). However, there are several serious limitations to this study. The report does not state the exact air concentration of 2,4,6-trinitrotoluene, the duration of exposure, or the number of exposed

workers with respiratory difficulties. It also does not specify the nature of those difficulties. The air concentration of 2,4,6-trinitrotoluene in the same plant was brought down to 0.3 mg/m^3 within a month, but there is no information on whether the respiratory difficulties disappeared or persisted in the affected workers (Morton et al. 1976).

No studies were located regarding respiratory effects in animals after inhalation exposure to 2,4,6-trinitrotoluene.

Hematological Effects. In England during World War I, there were numerous cases of anemia and some reports of fatal aplastic anemia among workers using 2,4,6-trinitrotoluene in the production of explosives (Hathaway 1985). Similar experiences were seen in other countries involved in the war. However, with the improvement of protective measures used during the manufacturing process, the number of cases of 2,4,6-trinitrotoluene toxicity has decreased dramatically. The results of 2,4,6-trinitrotoluene exposure on hemoglobin, hematocrit, and reticulocyte numbers are well documented. A dose-response relationship between 2,4,6-trinitrotoluene exposure and effects on the hematologic system was found in 626 workers exposed to 2,4,6-trinitrotoluene when they were compared with 865 nonexposed controls. Tests were taken over a 6-week period. However, the actual duration of the workers' exposures was not specified (Army 1976). The 2,4,6-trinitrotoluene estimated mean exposure levels ranged from <0.01 to 1.49 mg/m^3; this range includes exposures higher than the present TLV of 0.5 mg/m^3 (ACGIH 1993). Dose-related reductions in hemoglobin (9.9% lower than control) and hematocrit (11.6% lower that controls) and 50% higher reticulocyte counts were noted in exposed workers (Army 1976).

No abnormal values for hemoglobin were found in 43 workers employed in the manufacture of 2,4,6-trinitrotoluene who were monitored over a period of 5 months (Morton et al. 1976). During that time the air levels of 2,4,6-trinitrotoluene rose from 0.3 to 0.8 mg/m^3. This finding is indirectly supported by another occupational exposure study. Activities of two mitochondrial enzymes, δaminolevulinic acid synthase and heme synthase, were measured in reticulocytes in a chronic occupational exposure study of workers who developed cataracts (Savolainen et al. 1985). Mean 2,4,6-trinitrotoluene concentrations were 0-35 mg/m^3. Although the levels of the two enzymes were

lower in the exposed workers, none of them had clinical anemia. This finding indicates that 2,4,6-trinitrotoluene may not affect hemoglobin synthesis in the bone marrow and that possible effects on reticulocytes may occur in the circulation after the oxygenation of cells in the lungs has taken place.

Hyperplasia of the bone marrow is the first reaction of the hematopoietic system to 2,4,6-trinitrotoluene poisoning. The shaft of the femur and the ribs are usually filled with active red marrow. If the exposure to 2,4,6-trinitrotoluene continues, the bone marrow becomes hypocellular (Army 1978a).

No studies were located regarding hematological effects in animals after inhalation exposure to 2,4,6-trinitrotoluene.

Hepatic Effects. Toxic hepatitis has been the principal manifestation of 2,4,6-trinitrotoluene toxicity in humans (Army 1978a). During World War I, 2,4,6-trinitrotoluene production increased and many cases of toxic hepatitis were fatal (Army 1978a). Industrial hygiene techniques improved by World War II; consequently, both the number of toxic hepatitis cases and the number of fatalities due to 2,4,6-trinitrotoluene exposure decreased dramatically (Army 1978a).

A statistically significant increase in hepatic enzymes (serum glutamic-oxaloacetic transaminase [SGOT] and lactic dehydrogenase [LDH]) was noted in ammunition plant workers when the 2,4,6-trinitrotoluene level in the air increased from 0.3 to 0.8 mg/m^3 (during a 4-month period) at the same time that 2,4,6-trinitrotoluene production increased from 80% to 100% (Morton et al. 1976). Both these 2,4,6-trinitrotoluene air levels were close to the TLV of 0.5 mg/m^3 (ACGIH 1993). The SGOT increase was 20% above the maximal normal value (Morton et al. 1976). In the same group of workers, the LDH values increased from about 51 units to over 106, but since isoenzyme studies were not performed, it is difficult to say if this increase was due to liver toxicity or to hemolysis (hemoglobin levels decreased only slightly-see Hematological Effects above and Morten et al. 1976).

No significant differences in liver function were noted in a cross-sectional epidemiology study of 626 munitions workers from four plants exposed to 2,4,6-ttinitrotoluene when compared to 865 nonexposed controls (Army 1976). The majority of the workers were exposed to 0.5 mg/m^3 or less of 2,4,6-trinitrotoluene, and a few were exposed to 1.5 mg/m^3. No changes were noted in the other liver parameters evaluated in the study: LDH, bilirubin (total and direct), alkaline phosphatase, SGOT, and serum glutamic-pyruvic transaminase (SGPT) (Army 1976). One possible explanation for these findings is that exposure to 2,4,6-trinitrotoluene causes more liver toxicity in potentially susceptible workers, and that in some cases of long-term exposure, liver cells may adapt to moderate exposure levels (Hathaway 1985). A case-control study of Chinese workers exposed to 2,4,6-trinitrotoluene indicates that the likelihood of liver injury is increased among those workers who are heavy drinkers as compared to workers who are not heavy drinkers (Li et al. 1991). However, the parameters measured to arrive at the diagnosis of liver damage in these cases are not discussed.

No studies were located regarding hepatic effects in animals after inhalation exposure to 2,4,6-trinitrotoluene.

Dermal Effects. In an occupational exposure study, several of the workers handling 2,4,6-trinitrotoluene in an ammunition plant complained of dermatitis (Morton et al. 1976). Inhalation seemed to be the major route of exposure, although dermal exposure was possible. Therefore, the dermatitis cannot be attributed to either a local or a systemic effect with certainty. In addition, the study did not report the precise number of affected workers or the duration and level of exposure.

No studies were located regarding dermal effects in animals after inhalation exposure to 2,4,6-trinitrotoluene.

Ocular Effects. The appearance of cataracts is believed to be an effect of 2,4,6-trinitrotoluene exposure (Hathaway 1985; Savolainen et al. 1985) and is often associated with chronic exposures. Irreversible equatorial lens opacities/cataracts were reported in 6 out of 12 Finnish workers exposed to 2,4,6-trinitrotoluene for an average of 6.8 years (2.1-11.5 years of exposure) (Harkonen et al. 1983). The principal routes of exposure were probably inhalation and dermal, although this is not clearly

indicated in the report. Therefore, it is uncertain whether cataracts are a local or a systemic effect of 2,4,6-trinitrotoluene exposure. The opacities were detectable only on the periphery of the lens and appeared either continuous or discontinuous. The opacities of the lens were bilateral and symmetrical and did not affect visual fields or visual acuity. The workroom 2,4,6-trinitrotoluene air concentration was about 0.3 mg/m^3 with a range of 0.14-0.58 mg/m^3 (Harkonen et al. 1983). The progression of the cataract stops if the exposure to 2,4,6-trinitrotoluene stops. The mechanism of 2,4,6-trinitrotoluene cataract formation is not understood, but the possibility was raised that free radicals may play a role (Harkonen et al. 1983).

No studies were located regarding ocular effects in animals after inhalation exposure to 2,4,6-trinitrotoluene.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans or animals after inhalation exposure to 2,4,6-trinitrotoluene.

2.2.1.4 Neurological Effects

Very limited information is available regarding neurological effects in humans following inhalation exposure to 2,4,6-trinitrotoluene. Several workers who handled 2,4,6-trinitrotoluene in an ammunition plant reported altered taste, but no quantitative data are provided in the study (Morton et al. 1976). The concentration of 2,4,6-trinitrotoluene in the air was 0.3 mg/m^3, which was below the TLV of 0.5 mg/m^3 (ACGIH 1993). However, no details were provided on the exposure time or symptoms, so it is difficult to estimate the extent of the effect.

No studies were located regarding neurological effects in animals following inhalation exposure to 2,4,6-trinitrotoluene.

2.2.1.5 Reproductive Effects

A case-control study in two 2,4,6-trinitrotoluene plants in China indicated that 50 of the 104 workers that were examined for possible effects on semen had significantly lower semen volumes and a smaller percentage of motile spermatozoa, as well as a significantly higher incidence of sperm malformation, than the 33 controls (Li et al. 1993). Controls were clerks matched to the workers by income and by the city where they lived. However, exposure to 2,4,6-trinitrotoluene was not estimated. The only data available were annual measurements of air concentrations, so dose and effects cannot be correlated. Furthermore, confounding variables (beyond smoking and drinking) were not discussed. Possible important variables would include simultaneous exposures to other chemicals and heat in the workplace.

No studies were located regarding reproductive effects in animals after inhalation exposure to 2,4,6-trinitrotoluene.

2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to 2,4,6-trinitrotoluene.

2.2.1.7 Genotoxic Effects

The presence of mutagenic compounds in the urine of workers exposed to 2,4,6-trinitrotoluene was confirmed in two investigations (Ahlborg et al. 1985, 1988a). The initial study involved the screening of urine samples from 97 workers in a chemical plant producing pharmaceuticals and explosives (Ahlborg et al. 1985). Included in the study was a group of 14 individuals exposed to a maximum air concentration of 0.29 mg/m^3 2,4,6-trinitrotoluene. Urine samples were collected at the conclusion of a work shift, concentrated on XAD-2 resin, and assessed for mutagenic activity using *Salmonella typhimurium* TA98 and *Escherichia coli* WP2 uvrA in the presence or absence of exogenous metabolic activation. Baseline data for each participant were established from samples collected following a

4-week vacation. The excretion of genotoxic agents was indicated by a significant increase ($p<0.01$) in mutant colonies of strain TA98 without metabolic activation in the urine of workers exposed to 2,4,6-trinitrotoluene; baseline samples for this group were uniformly negative. The exclusion of smokers from the 2,4,6-trinitrotoluene exposure group did not alter these findings. Unmetabolized 2,4,6-trinitrotoluene was detected in the urine of workers with the highest level of mutagenic activity. The findings of this study are consistent with the demonstrated mutagenic activity of 2,4,6-trinitrotoluene in *S. typhimurium* TA98 without S9 activation (see Section 2.4).

In the follow-up study, urine samples from 50 individuals exposed to varying concentrations of 2,4,6-trinitrotoluene in the workplace were evaluated (Ahlborg et al. 1988a). Subjects were divided into three groups: no exposure (2,4,6-trinitrotoluene air concentrations were too low to be detected), mid-range exposure (0.1-0.3 mg/m^3), and high-range exposure (0.2-0.5 mg/m^3). For each individual, pre- and postexposure samples were collected, and health status data relative to smoking habits, alcohol consumption, diet, and medication were obtained. Samples were concentrated and assessed for mutagenic activity in *S. typhimurium* strain TA98 and a derivative of TA98 deficient in nitroreductase activity (TA98NR); the assays were conducted without exogenous metabolic activation. The concentrations of 2,4,6-trinitrotoluene and two major metabolites (4-aminodinitrotoluene [4-ADNT] and 2-aminodinitrotoluene [2-ADNT]) were also determined. In agreement with the earlier findings, evidence of mutagenic activity was present in the urine of groups exposed to 2,4,6-trinitrotoluene, but significant genotoxicity was confined to urine from individuals in the high exposure group. Strain TA98, rather than TA98NR, is the most sensitive indicator of induced gene mutations because of endogenous nitroreductase activity. The finding suggests that bacterial nitroreductase activity is the most probable primary cause of 2,4,6-trinitrotoluene-induced gene mutations. Although the relevancy of this finding to humans is not known, comparable nitroreductase activity may be present via intestinal microflora or mammalian cells. In contrast to the results of the earlier study (Ahlborg et al. 1985) in which detectable levels of 2,4,6-trinitrotoluene were found in the urine samples that exhibited the highest level of mutagenicity, no correlation between 2,4,6-trinitrotoluene concentration and mutagenesis was seen. However, a weak correlation was seen between the concentration of the major metabolite (4-ADNT) and mutagenesis. The study authors concluded that the wide variation in individual urine sample mutagenicity data in conjunction with toxicokinetics and individual rates of

2,4,6-trinitrotoluene uptake probably accounts for the lack of a correlation. Similarly, calculation of the uptake of 2,4,6-trinitrotoluene through inhalation based on air concentration provided much lower estimates than expected from the urine concentration of the metabolites. This finding indicates that dermal absorption may contribute significantly to total uptake.

No studies were located regarding genotoxic effects in animals after inhalation exposure to 2,4,6-trinitrotoluene.

Other genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

In a case of chronic occupational exposure to 2,4,6-trinitrotoluene, a 61-year-old male died of hepatocellular carcinoma (Garfinkel et al. 1988). Although he was exposed to 2,4,6-trinitrotoluene for 39 years as an ammunition plant worker, it is not known if 2,4,6-trinitrotoluene had a promoting role or any role in the development of the primary liver carcinoma. No discussion of exposure routes was included. In general, inhalation is assumed to be the primary pathway for worker exposure, but dermal contact and incidental ingestion due to hand-mouth contact cannot be ruled out.

No studies were located regarding cancer in animals after inhalation exposure to 2,4,6-trinitrotoluene.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to 2,4,6-trinitrotoluene. However, during World Wars I and II, many fatal cases of toxic jaundice and aplastic anemia occurred. The fatalities were attributed to 2,4,6-trinitrotoluene exposure during the manufacturing of munitions (Army 1978a; McConnell and Flinn 1946).

The concentrations at which 2,4,6-trinitrotoluene is acutely lethal in animals vary depending on the species and sex. Reported oral LD_{50} values for 2,4,6-trinitrotoluene are 1,010 and 1,320 mg/kg/day for male rats, and 795 and 820 mg/kg/day for female rats (Army 1978b; Dilley et al. 1982b). Acute oral LD_{50} values in male and female mice are 1,012 and 660 mg/kg/day, respectively. Doses were administered by gavage with oil as a vehicle (Army 1978b; Dilley et al. 1982b). The animals developed tremors, followed by mild convulsions, l-2 hours after exposure. In some animals, death occurred within 4 hours following the exposure. The animals that survived the convulsions were still alive 14 days after exposure (Dilley et al. 1982b).

2,4,6-Trinitrotoluene was found to be lethal to beagle dogs receiving 32 mg/kg/day orally (by capsule) for 26 weeks. One female dog died during week 16 after exhibiting considerable weight loss, diarrhea, and ataxia. A second female dog was sacrificed in a moribund state during week 14 of the study. The second female dog was observed to be dehydrated and emaciated, with low body temperature and signs of an advanced icteric state. No deaths occurred in male dogs (Levine et al. 1990b).

No deaths were observed in Fisher-344 rats fed 125 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks (Levine et al. 1990a). Similar observations were made in the chronic exposure studies. No changes in survival rates were seen in the same breed of rats that were fed 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a).

The LD_{50} values 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

No studies were located regarding respiratory, musculoskeletal, dermal, or ocular effects in humans or animals after oral exposure to 2,4,6-trinitrotoluene.

The highest NOAEL values and all reliable LOAEL values for each study and for each end point are recorded in Table 2-l and plotted in Figure 2-l.

TABLE 2-1 Levels of Significant Exposure to 2,4,6-Trinitrotoluene - 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 Sprague-Dawley	once (GO)				1320 M (LD50) 795 F (LD50)	Dilley et al. 1982b
2	Mouse NS	once (GO)				660 (LD50)	Dilley et al. 1982b
	Neurological						
3	Rat Sprague-Dawley	once (GO)				1320 M (convulsions, tremors) 795 F	Dilley et al. 1982b
4	Rat CD	4 d 2x/d (GO)		182 M			Short and Lee 1980
5	Mouse NS	once (GO)				660 (convulsions, tremors)	Dilley et al. 1982b
	INTERMEDIATE EXPOSURE						
	Death						
6	Dog Beagle	6 mo 1x/d (C)				32 F (2/6 dogs died)	Levine et al. 1990b
	Systemic						
7	Rat Sprague-Dawley	13 wk 1x/d (F)	Hemato	1.40	160 (moderate anemia and marked leukocytosis)		Dilley et al. 1982b
			Hepatic	34.7	160 (increased cholesterol)		
			Bd Wt	34.7	160 (significantly decreased ~15-20% body weight)		
			Other	6.97	34.7 (decreased food intake)		

TABLE 2-1 Levels of Significant Exposure to 2,4,6-Trinitrotoluene - 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 Wistar	6 wk 6d/wk (G)	Hepatic		200M (liver weight increased significantly)		Jiang et al. 1991
9	Rat Fisher 344	13 wk (F)	Hemato	5	25 (moderate anemia)		Levine et al. 1984
			Hepatic	5	25 (increased serum cholesterol levels)		
			Renal	25	125 (accumulation of yellow-brown pigmentation in the cortex)		
10	Rat Fisher 344	13 wk 1x/d (F)	Hemato	5	125M (increased reticulocytes; dose dependent anemia)		Levine et al. 1990a
			Hepatic	5	125 (increased liver weight and serum cholesterol)		
			Renal	5	125 (increased yellow-brown pigment in tubular epithelial cells of renal cortex)		
			Bd Wt	5		125 (significantly decreased body weight gain 60% to 75% of control)	
11	Mouse NS	13 wk 1x/d (F)	Hemato	35.7	193 (decreased RBC and hematocrit)		Dilley et al. 1982b
			Hepatic	35.7		193 (liver necrosis)	
12	Dog Beagle	13 wk 1x/d (F)	Gastro	2.0	20 (mucoid stools, diarrhea)		Dilley et al. 1982b
			Hemato	2.0	20 (moderate anemia)		
			Hepatic	2.0	20 (increased liver weight, bilirubin, and cholesterol)		

TABLE 2-1 Levels of Significant Exposure to 2,4,6-Trinitrotoluene - 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
13	Dog Beagle	6 mo 1x/d (C)	Gastro		0.5 (inflammation of small intestine)		Levine et al. 1990b
			Cardio	32			
			Hemato	2	8 M (hemolytic anemia, methemoglobinemia, and increased platelets)		
				8	32 F		
			Hepatic		0.5[b] (cloudy swelling hepatocytomegaly)	8 M (hepatic cirrhosis)	
			Ocular	32			
			Bd Wt	2	8 (statistically significant decrease in body weight 16.4%)		
	Immuno/Lymphoret						
14	Rat Sprague-Dawley	13 wk 1x/d (F)		34.7	160 (increased spleen weight, hemosiderosis, lymphocytosis)		Dilley et al. 1982b
15	Rat Fisher 344	13 wk (F)		25	125 (splenomegaly with moderate, diffuse sinusoidal congestion)		Levine et al. 1984
16	Rat Fisher 344	13 wk 1x/d (F)		5	125 (increased spleen weight with mild, diffuse sinusoidal congestion)		Levine et al. 1990a
17	Mouse NS	13 wk 1x/d (F)		35.7 M	193M (increased spleen weight hemosiderosis, lymphopenia)		Dilley et al. 1982b

TABLE 2-1 Levels of Significant Exposure to 2,4,6-Trinitrotoluene - Oral (continued)

Key to figure [a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
18	Dog Beagle	13 wk 1x/d (F)		2.0	20 (increased spleen weight and globulin levels, splenic hemosiderosis)		Dilley et al. 1982b
19	Dog Beagle	6 mo 1x/d (C)		2	8M (enlarged spleen)		Levine et al. 1990b
	Neurological						
20	Rat Fisher 344	13 wk (F)		25	125 (slight lethargy and ataxia)	300 (brain lesions: focal vacuolation and/or malacia of cerebellar folia)	Levine et al. 1984
21	Dog Beagle	13 wk 1x/d (F)		2.0	20M (inactivity)		Dilley et al. 1982b
22	Dog Beagle	6 mo 1x/d (C)		8	32 (slight ataxia)		Levine et al. 1990b
	Reproductive						
23	Rat Sprague-Dawley	13 wk 1x/d (F)		34.7		160 M (testicular atrophy, hyperplasia of interstitial cells, atrophy of the epididymis)	Dilley et al. 1982b
24	Rat Wistar	6 wk 6d/wk (G)				200 M (significantly decreased testes weight)	Jiang et al. 1991
25	Rat Wistar	6 wk 6d/wk (G)			200M (decreased serum ceruloplasmin and decreased serum zinc levels at six weeks)		Jiang et al. 1991

TABLE 2-1 Levels of Significant Exposure to 2,4,6-Trinitrotoluene - Oral (continued)

Key to figure [a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
26	Rat Fisher 344	13 wk (F)		25		125 M (atrophic seminiferous tubules, degenerated germinal epithelium)	Levine et al. 1984
27	Rat Fisher 344	13 wk 1x/d (F)		5		125 M (degenerated germinal epithelium)	Levine et al. 1990a
	CHRONIC EXPOSURE						
	Systemic						
28	Rat Fischer 344	24 mo. (F)	Hemato	10 F	50 F (bone marrow fibrosis)		Army 1984a
			Hepatic	2	10 (increased cholesterol levels, hepatomegaly, hepatocellular hyperplasia)		
			Renal	2	10 (increase in pigment in epithelial cells of proximal convoluted tubules)		
			Bd Wt	2	10 (14% decrease in body weight gain)		
29	Mouse B6C3F1	24 mo. (F)	Hemato	10	70 (mild anemia)		Army 1984b
			Hepatic	10	70 (increased liver weight, hypotriglyceridemia, reduced serum globulin levels)		
			Bd Wt	1.5	10 (10-15% decrease in body weight gain)		

TABLE 2-1 Levels of Significant Exposure to 2,4,6-Trinitrotoluene - 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
	Immuno/Lymphoret						
30	Rat Fisher 344	24 mo. (F)		2	10 (sinusoidal congestion, extramedullary hematopoiesis, hemosiderin-like pigment)		Army 1984a
31	Mouse B6C3F1	24 mo. (F)		10 F 70 M	70 F (enlargement of the spleen and lymph nodes)		Army 1984b
	Cancer						
32	Rat Fisher 344	24 mo (F)				50 F (CEL- 22% females with carcinoma and 9% with papilloma of urinary bladder)	Army 1984a
33	Mouse B6C3F1	24 mo (F)				1.5 F (CEL-28% females with leukemia and/or lymphoma of spleen)	Army 1984b

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

[b]Used to derive intermediate oral Minimal Risk Level (MRL); dose divided by an uncertainty factor of 1000 (10 for extrapolation for animals to humans, 10 for human variability, and 10 for use of a LOAEL) resulting in an MRL of 0.0005 mg/kg/day.

(C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); (F) = feed; F = females; (G) = gavage; Gastro = gastrointestinal; (GO) = gavage in oil; Hb = hemoglobin; Hct = hematocrit; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = males; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; RBC = red blood cell; SGPT = serum glutamic pyruvate transaminase; wk = week(s); x = time(s); < = decrease; > = increase

Figure 2-1. Levels of Significant Exposure to 2,4,6-Trinitotoluene – Oral

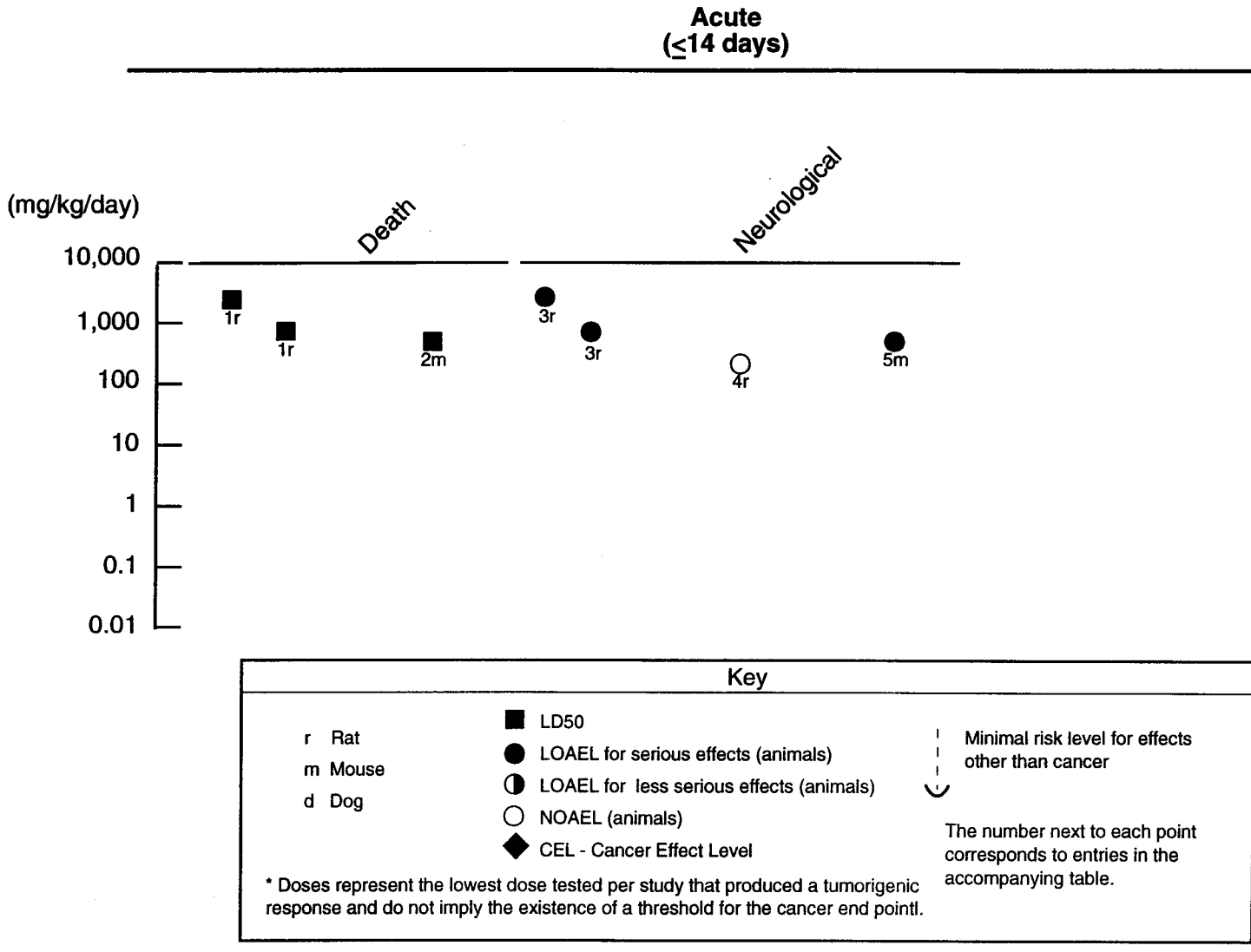


Figure 2-1. Levels of Significant Exposure to 2,4,6-Trinitotoluene – Oral (continued)

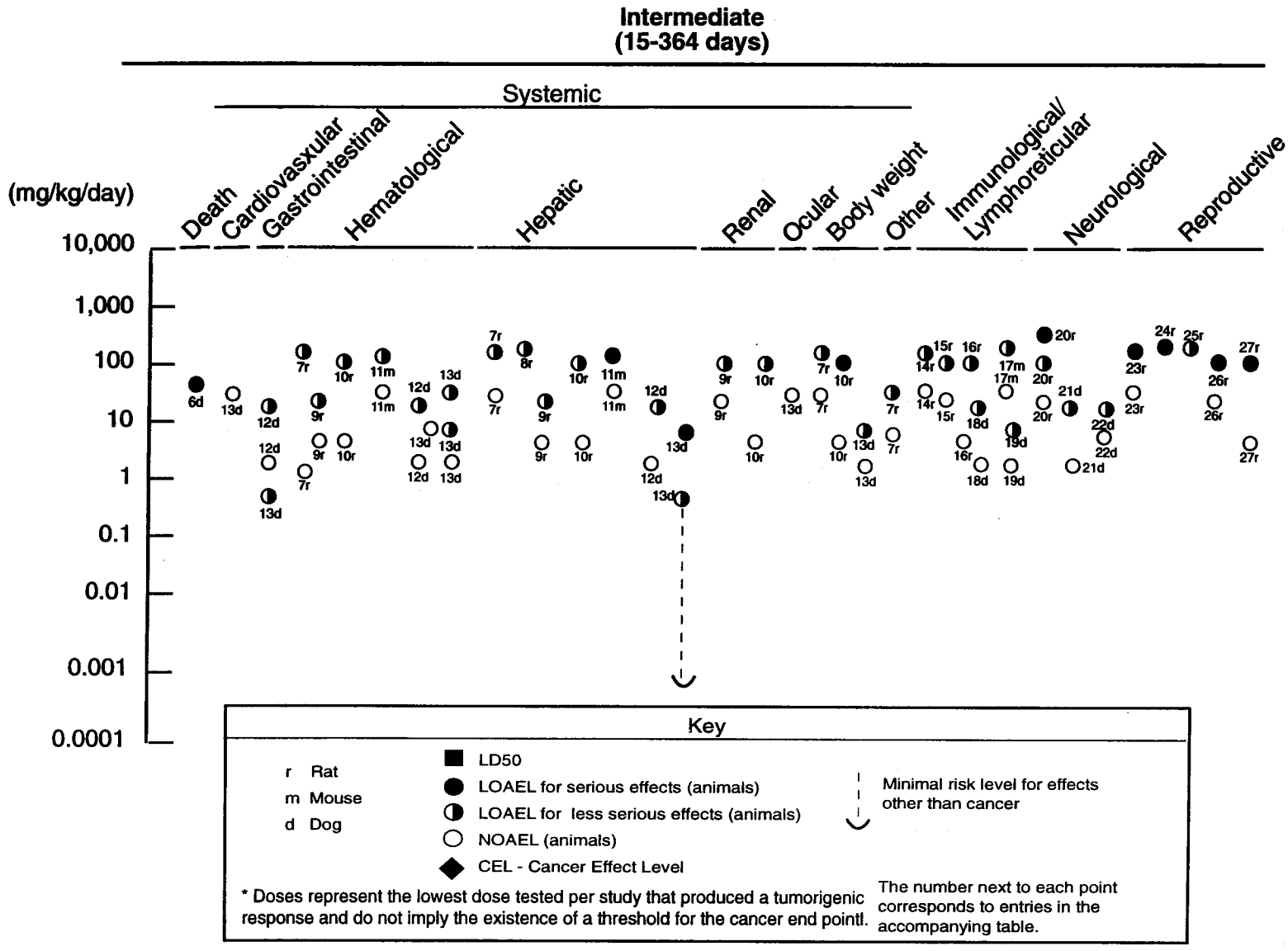
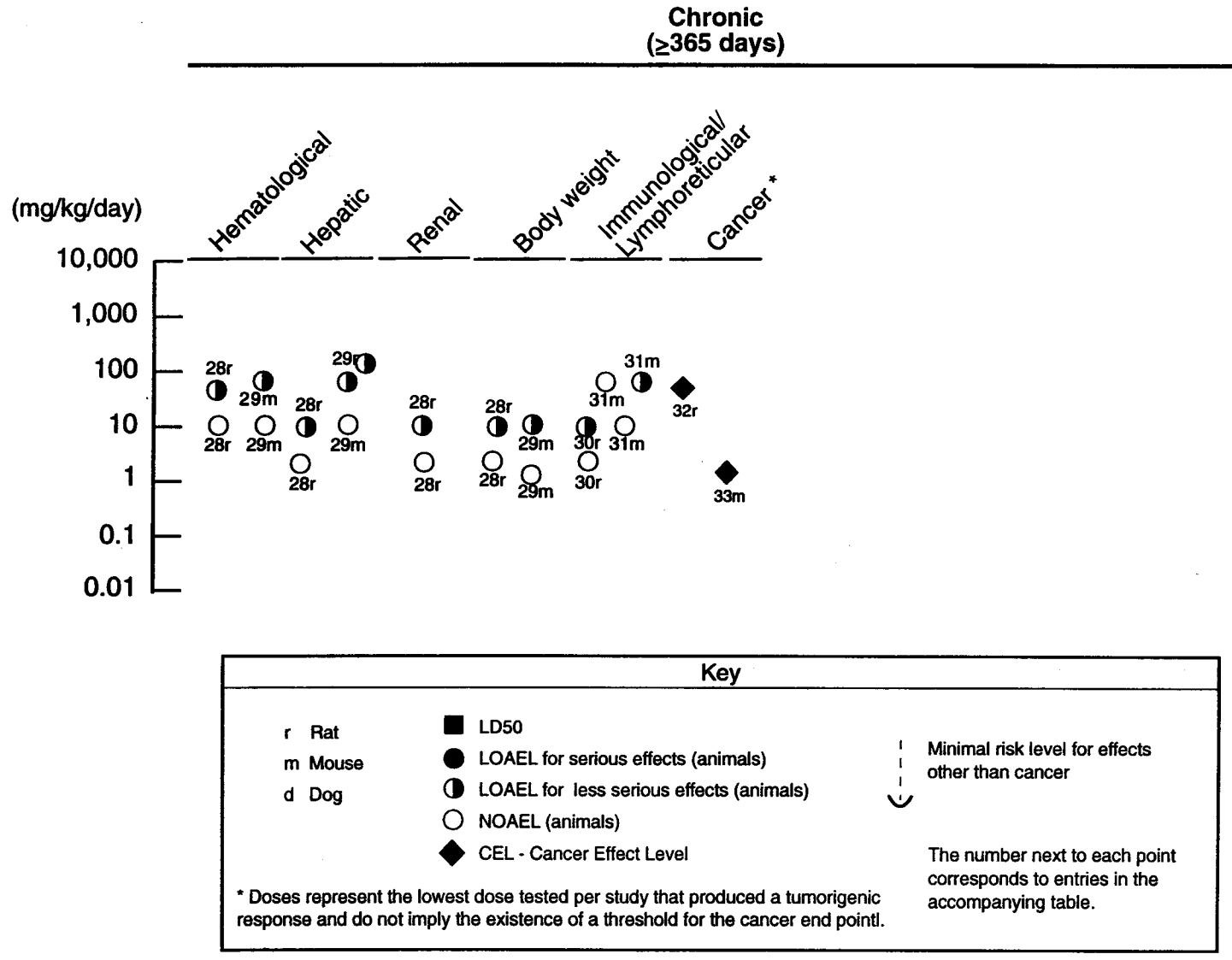


Figure 2-1. Levels of Significant Exposure to 2,4,6-Trinitrotoluene – Oral (continued)



Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to 2,4,6-trinitrotoluene.

Very limi.ted information is available regarding cardiovascular effects in animals after oral exposure to 2,4,6-trinitrotoluene. Intermediate exposure of beagle dogs to a 2,4,6-trinitrotoluene dose as high as 32 mg/kg/day for 26 weeks did not cause any changes in electrocardiogram results or heart rates (Levine et al. 1990b).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in, humans after oral exposure to 2,4,6-trinitrotoluene.

Adverse gastrointestinal effects were reported in dogs after intermediate oral exposure to 2,4,6-trinitrotoluene. Dogs receiving 20 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks had mucoid stools and diarrhea (Dilley et al. 1982b). In another longer intermediate exposure study, histopathology revealed inflammation of a part of the small intestine in beagle dogs fed 0.5, 2, 8, or 32 mg/kg/day 2,4,6-trinitrotoluene for 6 months. Although not dose related, the observed enteritis was more frequent in dogs treated with the highest dose of 2,4,6-trinitrotoluene. None of the control animals had enteritis (Levine et al. 1990b).

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to 2,4,6-trinitrotoluene.

Anemia is one of the frequent signs of 2,4,6-trinitrotoluene toxicity. Adverse effects on standard hematologic parameters were observed in rats (Dilley et al. 1982b; Jiang et al. 1991; Levine et al. 1984, 1990a), mice (Dilley et al. 1982b), and dogs (Dilley et al. 1982b; Levine et al. 1990b) after intermediate oral exposures to 2,4,6-trinitrotoluene. Compensatory responses occurring as a result of anemia (including reticulocytosis, macrocytosis, and increased levels of nucleated erythrocytes) were observed in Fischer-344 rats fed 125 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks (Levine et al. 1990a). Dose-related anemia was also observed in Fischer-344 rats fed 10 or 50 mg/kg/day

2,4,6-trinitrotoluene for 24 months (Army 1984a). In this chronic exposure study, both male and female rats had reduced hematocrit, hemoglobin, and red blood cells. These hematological effects were observed throughout the entire duration of the study in male rats, but only for the first year in female rats. Methemoglobin was observed in male rats at doses of 10 and 50 mg/kg/day. Howell-Jolley and Heinz Bodies occurred at 50 mg/kg/day in male rats. Therefore, male rats seemed somewhat more sensitive than female rats. Reticulocytosis, but not macrocytosis, was present as a compensatory response to the anemic state in all animals. Histopathology revealed splenic lesions consisting of sinusoidal congestion, extramedullary hematopoiesis, and increased amounts of hemosiderin-like pigment (Army 1984a). The findings are consistent with the hypothesis that 2,4,6-trinitrotoluene induces anemia by causing hemolysis through oxidative damage which is mediated by 2,4,6-trinitrotoluene and/or its metabolites. This conclusion is further supported by the presence of methemoglobinemia, produced by the oxidation of the heme iron, observed when 2,4,6-trinitrotoluene was fed to rats at 300 mg/kg/day for 13 weeks (Levine et al. 1984), to rats at 10 or 50 mg/kg/day for 24 months (Army 1984a), and to dogs at 32 mg/kg/day for 6 months (Levine et al. 1990b). Mild anemia was also noted in $B6C3F_1$ mice fed 70 mg/kg/day 2,4,6-trinitrotoluene for 24 months (Army 1984b). Anemia, as indicated by dose-dependent decreases in hematocrit and hemoglobin levels and decreased erythrocyte counts, was observed in dogs administered 2,4,6-trinitrotoluene (via capsules) for 6 months (Levine et al. 1990b). The anemia was compensated by reticulocytosis, macrocytosis, and an increased number of nucleated erythrocytes. There was an elevated level of methemoglobin in all dogs treated with 32 mg/kg/day of 2,4,6-trinitrotoluene. No effects on methemoglobin levels, or blood cells and Heinz body counts were found in monkeys following gavage administration of 2,4,6-trinitrotoluene at dose levels as high as 1.0 mg/kg/day (Martin and Hart 1974).

Bone marrow fibrosis was present in a significant number of female rats fed 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a).

Dogs treated daily with 8 mg/kg/day of 2,4,6-trinitrotoluene for 6 months had approximately a 68% and 22% increase in platelet levels over the control animals for males and females respectively (Levine et al. 1990b). A similar increase was noted in Fischer-344 rats treated with 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a). This increase occurred during the 2nd year of treatment and was not present at the end of the study period, week 104 (Army 1984a). Although the increase in

the number of platelets appeared to be related to 2,4,6-trinitrotoluene treatment, its significance is not clear.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to 2,4,6-trinitrotoluene. However, toxic jaundice was often fatal in workers exposed to 2,4,6-trinitrotoluene in ammunition plants during World War I (Army 1978a). Between 1916 and 1941, 475 cases of toxic jaundice were recorded in a British ammunition plant; 125 of these were fatal (Army 1978a). During World War II, only eight fatal cases of toxic hepatitis were recorded in the United States because industrial hygiene techniques had improved since the first world war (Army 1978a).

Limited information is available for adverse hepatic effects in animals after acute oral exposure to 2,4,6-trinitrotoluene. The most common adaptive change observed in mice, rats, and dogs during intermediate exposure was an increase in liver weight and/or size. A significant increase in liver weight was noted in rats receiving 200 mg/kg/day of 2,4,6-trinitrotoluene for 6 weeks (Jiang et al. 1991) or 125 mg/kg/day for 13 weeks (Levine et al. 1984). A similar observation was made in male mice treated with 193 mg/kg/day for 13 weeks (Dilley et al. 1982b). Dogs treated with 20 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks also had increased liver weight (Dilley et al. 1982b). Another adaptive response to 2,4,6-trinitrotoluene-induced hepatotoxicity was a reduction in serum glutamicpyruvic transaminase (SGPT) in rats treated with 160 mg/kg/day and dogs treated with 20 mg/kg/day for 13 weeks (Dilley et al. 1982b). No change in serum glutamic-oxaloacetic transaminase (SGOT) was noted in those same animals (Dilley et al. 1982b). Dose-related changes such as hepatocytomegaly and cloudy swelling were present in dogs after exposure to doses of 0.5 mg/kg/day or greater of 2,4,6-trinitrotoluene for 6 months (Levine et al. 1990b). In addition, a reduction in SGPT activity was noted in dogs administered 8 or 32 mg/kg/day. Necrotic lesions in the liver were found in mice treated with 193 mg/kg/day for 13 weeks (Dilley et al. 1982b), while hepatic cirrhosis was seen in dogs treated with 8 mg/kg/day of 2,4,6-trinitrotoluene for 6 months (Levine et al. 1990b). Monkeys administered 1 mg/kg/day 2,4,6-trinitrotoluene (by gavage) for 90 days displayed ironpositive material in the liver (Martin and Hart 1974). However, results of the bromosulfophthalein (BSP) dye test revealed no effects on liver function. This study was limited because only three monkeys per sex per were used, precluding statistical analysis of the data. In addition, there was a

high frequency of emesis in treated animals. These results indicate that there is a species difference regarding 2,4,6-trinitrotoluene hepatotoxicity. Dogs seem to respond to lower concentrations of 2,4,6-trinitrotoluene than do mice or rats.

The hepatotoxicity of 2,4,6-trinitrotoluene was reflected in elevated levels of cholesterol present in the serum after intermediate and chronic exposures. Increased serum cholesterol levels were present in rats treated with 25 (Levine et al, 1984), 125 (Levine et al. 1990a), and 160 mg/kg/day (Dilley et al. 1982b) of 2,4,6-trinitrotoluene for 13 weeks. A similar finding was seen in dogs fed 20 mg/kg/day of 2,4,6-trinitrotoluene for the same time period (Dilley et al. 1982b). Increased cholesterol levels were also found in male and female rats treated with 50 mg/kg/day for 24 months (Army 1984a).

Evidence for adverse hepatic effects has also been revealed in studies of chronic exposure. Doserelated hepatomegaly resulting from hepatocellular hyperplasia was observed in Fischer-344 rats given 10 or 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a).

Serum lipids were affected by chronic administration of 2,4,6-trinitrotoluene to rats (Army 1984a). The levels of serum triglycerides were affected differently in male and female rats exposed to 2, 10, or 50 mg/kg/day of 2,4,6-trinitrotoluene (Army 1984a). Treatment-related hypotriglyceridemia was observed in females at 2 mg/kg/day; this change was statistically significant at 10 and 50 mg/kg/day during week 104 of treatment. In male rats treated with 50 mg/kg/day, a significant reduction in triglyceride levels was seen after 104 weeks of treatment (Army 1984a). The results indicate that female rats were more susceptible to 2,4,6-trinitrotoluene-induced reduction in serum triglyceride levels than male rats that showed reduced triglycerides only at the highest treatment doses. Hypoglyceridemia was also observed in mice treated with 70 mg/kg/day 2,4,6-trinitrotoluene for 24 months (Army 1984b), and a decrease in glucose levels was observed in dogs treated with 8 or 32 mg/kg/day 2,4,6-trinitrotoluene (Levine et al. 1990b).

Jaundice (icterus) was observed in beagle dogs treated with 32 mg/kg/day for 26 weeks (Levine et al. 1990b). The presence of jaundice was evidenced by elevated bilirubin levels in both serum and urine and increased urobilinogen values (Levine et al. 1990b). Histopathological analysis of these animals

revealed hemosiderosis in Kupffer's cells in all dogs receiving 8 and 32 mg/kg/day and in one female receiving 2 mg/kg/day for 26 weeks (Levine et al. 1990b).

One of the proposed mechanisms of 2,4,6-trinitrotoluene-induced toxicity is an increase in free radical levels which occurs after exposure. Superoxide radicals and hydrogen peroxide were measured in mitochondria and microsomes from livers of monkeys treated with 0, 60, or 120 mg/kg/day of 2,4,6-trinitrotoluene for 12 weeks (Kong et al. 1989). The amount of superoxide radicals was indirectly measured by the formation of adrenochrome from adrenalin, and hydrogen peroxide production was evaluated by the conversion of methanol to formaldehyde. There was a dose-dependent increase in superoxide radicals and hydrogen peroxide production in liver mitochondria and microsomes (Kong et al. 1989). These findings were confirmed when mitochondria and microsomes obtained from various organs were treated in vitro with 0, 0.04, 0.2, or 1 mmol of 2,4,6-trinitrotoluene and then tested for adrenochrome and formaldehyde production. Different amounts of hydrogen peroxide were produced in the mitochondria of the various organs. The highest amount of hydrogen peroxide was present in the liver followed by brain, testicle, kidney cortex, and kidney medulla (Kong et al. 1989).

Renal Effects. Discoloration of the urine is among the first indications of 2,4,6-trinitrotoluene intoxication in humans. The color of urine ranges from abnormal amber to a deep red, and in most cases the results are positive for Webster's test (a qualitative urine test for 2,4,6-trinitrotoluene based on the formulation of purple color in acidified urine samples following extraction with ether and treatment with potassium hydroxide) (Army 1978a).

Accumulation of yellowish-brown pigment in the renal cortex of rats treated with 125 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks was seen during histopathological analysis. The authors suggest that this pigment may have represented known photolytic decomposition products of 2,4,6-trinitrotoluene (Levine et al. 1984, 1990b). The same observation was made in female rats treated with 10 or 50 mg/kg/day for 24 months (Army 1984a). A dose-related increase in granular pigment within the cytoplasm of epithelial cells of proximal convoluted tubules was observed at the end of the treatment period in male rats receiving either 10 or 50 mg/kg/day for 24 months (Army 1984a). Increased filtration rate was also present in these chronically exposed animals (Army 1984a).

2.2.2.3 Immunological and Lymphoreticular Effects

Limited information was located regarding immunological effects in humans after oral exposure to 2,4,6-trinitrotoluene. However, an early reaction to 2,4,6-trinitrotoluene intoxication was an increase in the number of mononuclear leukocytes found in the blood counts of 105 exposed individuals (Army 1978a). This increase seems to precede any other symptom and remains positive for 2-3 months; therefore, it would be helpful in the differential diagnosis of 2,4,6trinitrotoluene poisoning, especially when Webster's test is negative (Army 1978a). The route of exposure and precise dose were not defined in this report.

An increase in lymphocyte numbers was also seen in nine fatal cases of 2,4,6-trinitrotoluene toxicity in humans. The normal range for lymphocytes is 20-40% of the total white blood cell count. In the affected patients, the average was 78% of the total white blood count (range, 61-92%) (Army 1978a). Patients who recovered after 2,4,6-trinitrotoluene intoxication had lymphocyte counts which were approximately 46% of the total white cell count (Army 1978a).

No adverse lymphoreticular effects as assessed by changes in lymphocyte levels and histological changes in the spleen were seen in dogs treated with 2.0 mg/kg/day, rats treated with 34.7 mg/kg/day, or mice treated with 35.7 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks (Dilley et al. 1982b).

Increased spleen weight was seen in mice, rats, and dogs after an exposure of 13 weeks to 2,4,6-trinitrotoluene. The splenomegaly is possibly related to an increased clearance of hemolysed cells (Dilley et al. 1982b; Levine et al. 1984, 1990a). Male and female dogs exposed to 20 mg/kg/day, mice exposed to 193 mg/kg/day (Dilley et al. 1982b), and rats exposed to 125 mg/kg/day (Levine et al. 1984, 1990a) and 160 mg/kg/day (Dilley et al. 1982b) all had increased spleen weights after 13 weeks of exposure. Spleen enlargement related to 2,4,6-trinitrotoluene administration was also noted in dogs treated with 8 or 32 mg/kg/day for 26 weeks. The ratios of myeloid to erythroid cells were also significantly lower in these dogs, and they had varying degrees of splenic congestion (Levine et al. 1990b). Splenic hemosiderosis related to 2,4,6-trinitrotoluene treatment was also present in rats receiving 160 or 300 mg/kg/day (Dilley et al. 1982b; Levine et al. 1984), mice receiving 193 mg/kg/day (Dilley et al. 1982b), and dogs receiving 20 mg/kg/day (Dilley

et al. 1982b) of 2,4,6-trinitrotoluene for 13 weeks. Lymphopenia was present in mice treated with 193 mg/kg/day for 13 weeks (Dilley et al. 1982b). Increased globulin levels and leukocytosis were noted in the dogs treated with 20 mg/kg/day after a 4 week recovery period. Leukocytosis was observed in rats fed 160 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks (Dilley et al. 1982b). Sinusoidal congestion, extramedullary hematopoiesis, and hemosiderin-like pigment in the spleen were observed in male and female rats fed 10 or 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a). Enlargement of the spleen and lymph nodes was noted in female mice treated with 70 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984b).

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to 2,4,6-trinitrotoluene.

In acute exposure studies, rats fed 182 mg/kg/day of 2,4,6-trinitrotoluene for 4 days showed no signs of neurotoxicity as measured by changes in zoxazolamine paralysis time and hexobarbital sleeping time (Short and Lee 1980). However, in a single-dose oral LD_{50} study in rodents, rats and mice showed signs of inactivity, were tremulous, developed convulsions, and died (Dilley et al. 1982b).

Similar observations were made in intermediate-duration studies. No signs of neurotoxicity were seen after 13 weeks of 2,4,6-trinitrotoluene treatment in dogs receiving 0.2 mg/kg/day (Dilley et al. 1982b), monkeys receiving 1 mg/kg/day (Martin and Hart 1974), or rats receiving 1.42 mg/kg/day (Dilley et al. 1982b). Dogs treated with 32 mg/kg/day for 6 months were ataxic (Levine et al. 1990b), while inactivity was observed in dogs after treatment with 20 mg/kg/day for 13 weeks (Dilley et al. 1982b). Dose-related changes in behavior such as lethargy and/or ataxia were seen in rats treated with 34.7 or 125 mg/kg/day for 13 weeks (Dilley et al. 1982b; Levine et al. 1984). Brain lesions with focal vacuolation were seen in rats receiving 300 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks (Levine et al. 1984).

No significant signs of neurotoxicity were seen in Fischer-344 rats treated with up to 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a). The combined results of acute and intermediate

exposure studies indicate species differences in 2,4,6-trinitrotoluene-induced neurotoxicity, with dogs being more sensitive than rats or mice.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to 2,4,6-trinitrotoluene.

Significantly decreased testes weights and testes zinc and copper concentrations were observed in male rats exposed to 200 mg/kg/day 2,4,6-trinitrotoluene for 6 weeks (Jiang et al. 1991). In addition, the serum ceruloplasmin concentration was significantly lower in the male rats. Zinc metabolism seems to be affected more than copper metabolism by 2,4,6-trinitrotoluene treatment. Although there was a close correlation between testicular weight and testicular zinc concentration, the role of zinc in decreasing testicular weight is not clear (Jiang et al. 1991). However, zinc is known to be essential for maintenance of normal testicular function (Jiang et al. 1991). No adverse reproductive effects were noted in rats exposed to 1.42 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks (Dilley et al. 1982b). However, male rats treated with 125, 160, or 300 mg/kg/day for the same period of time had serious reproductive effects such as degenerated germinal epithelium, testicular atrophy, and atrophic seminiferous tubules (Dilley et al. 1982b; Levine et al. 1984, 1990a). Testicular atrophy was not reversible in rats allowed 4 weeks of recovery (Dilley et al. 1982b).

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after oral exposure to 2,4,6-trinitrotoluene.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after oral exposure to 2,4,6-trinitrotoluene

Results were negative from in viva studies employing the oral route of exposure to ascertain whether 2,4,6-trinitrotoluene has the potential to induce clastogenic effects in somatic cells or increase the frequency of unscheduled deoxyribonucleic acid (DNA) synthesis (UDS) in liver cells. However, the findings from somatic cell cytogenetic assays with rats were compromised and, therefore, do not fully support a negative conclusion.

In the bone marrow test, groups of five male Sprague-Dawley rats were administered dietary concentrations of 0.002% or 0.25% 2,4,6-trinitrotoluene for 28 days; two additional groups of five rats each were similarly treated and allowed a 28-day recovery period (Army 1978c). At the conclusion of the treatment or recovery period, animals were sacrificed; bone marrow cells were harvested and examined for abnormal chromosome morphology. No animals died prior to the scheduled sacrifice. The study authors attributed the reduced body weight observed in the high-dose group to the palatability of the test material rather than to a toxic effect. The slight depression in the mitotic indices for high-dose animals at the conclusion of treatment was not considered indicative of a cytotoxic effect on the target organ (bone marrow cells). Although no chromosome aberrations were scored in the exposure groups immediately after treatment or following the 28-day recovery period, the failure to demonstrate overt toxicity in the test animals or cytotoxic effects on the target organ renders the study insufficient to fully support the conclusion that 2,4,6-trinitrotoluene was negative in this in vivo cytogenetics assay.

2,4,6-Trinitrotoluene was administered by oral gavage at doses of 100, 200, 500, or 1,000 mg/kg to male Alderley Park rats and at doses of 200, 500, and 1,000 mg/kg to male Fischer-344 rats (up to three rats/group/strain) to investigate UDS in liver cells (Ashby et al. 1985). There was no evidence of a cytotoxic or genotoxic effect on the hepatocytes of either strain 12 hours after 2,4,6-trinitrotoluene exposure.

Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to 2,4,6-trinitrotoluene. However, a preliminary study of a German population living near the sites of two World War II munitions plants indicates an association between increased rates of some types of leukemia and living in a town near 2,4,6-trinitrotoluene waste from these plants (Kolb et al. 1993). The study shows increased relative risk of acute myelogenous leukemia (AML) for adult males and females living near the former explosives plants when compared with adults in a neighboring county. The relative risk is particularly high for individuals over 65 years of age. However, study case numbers are very small. The relative risk for chronic myelogenous leukemia (CML) is also increased for males but there was only one case among females so comparisons could not be made. The relative proximity of the cases of leukemia to the sites of 2,4,6-trinitrotoluene manufacture or disposal is not known, nor are any 2,4,6-trinitrotoluene concentrations in the environment reported. No investigation of confounding variables (i.e., benzene exposure or occupational exposure to carcinogens) has been done. The study concludes that a causal relationship is suggested, but further investigation of the living and working conditions of the populations is required (Kolb et al. 1993).

In a chronic study, groups of 150 (75 males and 75 female) Fischer-344 rats were exposed to 0, 0.4, 2.0, 10.0, and 50.0 mg/kg/day of 2,4,6-trinitrotoluene in their food for 24 months (Army 1984a). A statistically significant number of female rats (12/55 or 21.8%) exposed to 50-mg/kg/day doses developed urinary bladder carcinomas. Urinary bladder papillomas were present in 1/55 and 5/55 female rats fed 10 and 50 mg/kg/day, respectively. No metastases were observed in any of the animals. Histopathologic lesions included increased incidence of hyperplastic, preneoplastic, and neoplastic changes of the mucosal epithelium of the urinary bladder. The cancer incidence observed in this chronic exposure study is further supported by renal and urinary bladder hyperplasia observed in treated animals. None of the control animals developed lesions of the urinary bladder. In a similar study conducted in groups of 150 B6C3F_1 mice (75 males and 75 females), a statistically significant incidence ($p<0.01$) of leukemia and/or malignant lymphoma of the spleen was present in female mice receiving 70 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984b). Leukemia and/or malignant lymphoma of the spleen was noted in 28% and 32% of the female mice administered 1.5 and 10 mg/kg/day, respectively. The increase in the cancer incidence in the female mice receiving 1.5

or 10 mg/kg/day of 2,4,6-trinitrotoluene was not statistically significant. Histopathology revealed that leukemia was of granulocytic or lymphocytic type, while lymphoma was histiocytic, lymphocytic, or of a mixed type. All the lesions were treatment related and systemic in nature. The neoplasias involved other organs and tissues such as adrenals, bone marrow, brain, gastrointestinal tract, eyes, kidneys, liver, lungs, and lymph nodes. The occurrence of combined leukemia/malignant lymphoma seemed to be dose related but was not statistically significant. Based on the information from these two chronic animal studies, EPA has classified 2,4,6-trinitrotoluene as a possible human carcinogen (Group C) (EPA 1989b).

2.2.3 Dermal Exposure

In many occupational studies, it is often difficult to make a definitive distinction between dermal and inhalation exposures, as was indicated in the section on inhalation exposure. Therefore, some of the findings described in the inhalation section will be repeated in this section.

2.2.3.1 Death

Adverse effects of 2,4,6-trinitrotoluene on the liver and hematopoietic system have caused the greatest number of deaths among munitions workers. There were 475 deaths out of about 17,000 cases of 2,4,6-trinitrotoluene poisoning in the United States within 7.5 months during World War I (McConnell and Flinn 1946). In the same report, 22 cases of death due to occupational exposure to 2,4,6-trinitrotoluene during World War II were described (McConnell and Flinn 1946). In this series of fatal cases, 8 died from toxic hepatitis, 13 died from aplastic anemia, and 1 died probably from the combination of both these conditions (McConnell and Flinn 1946). The authors indicated that exposure occurred by dermal contact and inhalation.

No studies were located regarding death in animals after dermal exposure to 2,4,6-trinitrotoluene.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, or musculoskeletal effects in humans or animals after dermal exposure to 2,4,6-trinitrotoluene.

Hematological Effects. Acute hemolytic disease was described in three ammunition plant workers who filled shells with 2,4,6-trinitrotoluene (Djerassi and Vitany 1975). All three were deficient in glucose-6-phosphate dehydrogenase (G6PD), an enzyme that catalyzes the oxidation of glucose 6-phosphate to 6-phosphoglucono-lactone. All three cases developed acute severe hemolysis 2-3 days after being exposed to 2,4,6-trinitrotoluene and had very similar symptoms: paleness, weakness, and vertigo. They also had decreased hemoglobin levels, decreased hematocrit, and increased reticulocyte numbers (Djerassi and Vitany 1975). All three recovered and had no further complications when examined 5 and 10 years later. Limitations of this report are that the routes of exposure are not specified, although it seems that there was dermal and inhalation exposure, and that the level of exposure is not known. However, the authors note that the air levels of 2,4,6-trinitrotoluene were higher than the allowed daily exposure limit, which was 1.5 mg/m^3 at that time.

No studies were located regarding hematological effects in animals after dermal exposure to 2,4,6-trinitrotoluene.

Hepatic Effects. A modified MacLagen test (thymol turbidity test) was used to give evidence of cirrhosis or hepatitis resulting from 2,4,6-trinitrotoluene exposure in ammunition workers (Goodwin 1972). In a retrospective study spanning 20 years, the data showed that 40 out of 4,641 workers had >5 MacLagen units (2.9 MacLagen units is considered to be normal); the length of exposure and dose of 2,4,6-trinitrotoluene were not specified. However, the hepatotoxicity was reversible. All the workers with a MacLagen test result of >5 units were transferred to other jobs, and their readings returned to normal within 3 weeks (Goodwin 1972).

No studies were located regarding hepatic effects in animals after dermal exposure to 2,4,6-trinitrotoluene.

Renal Effects. No adverse renal effects were reported in humans after acute exposure to 2,4,6-trinitrotoluene as measured by Webster's reaction and the aminodinitrotoluene (ADNT) test (Hassman and Hassmanova 1976). In this study, the exposure route was not clearly specified, but it seems that dermal contact and inhalation were the major routes of exposure.

No studies were located regarding renal effects in animals after dermal exposure to 2,4,6-trinitrotoluene.

Dermal Effects. Allergic contact dermatitis with erythema (Goh 1988) and erythematous papillar rush with edema (Goh and Rajan 1983) were reported in two ammunition workers after intermediate exposures to 2,4,6-trinitrotoluene. In both cases, patch tests were used to determine the allergen. The results showed that 5% is the most suitable concentration for patch testing in order to avoid false positive or negative results (Goh and Rajan 1983). In both affected workers, dermal reactions developed on parts of the body that were exposed and in direct contact with 2,4,6-trinitrotoluene, such as hands and forearms. These reactions subsided and disappeared when the workers were transferred to different jobs and were no longer in contact with 2,4,6-trinitrotoluene.

No studies were located regarding dermal effects in animals after dermal exposure to 2,4,6-trinitrotoluene.

Ocular Effects. The development of cataracts in humans is believed to be specific to 2,4,6-trinitrotoluene exposure (Hathaway 1985) and is often associated with chronic exposures. Equatorial lens opacities/cataracts were reported in 6 out of 12 Finnish workers (mean age, 39.5±8.9 years) exposed to 2,4,6-trinitrotoluene for an average of 6.8±4.7 years (Harkonen et al. 1983). The principal routes of exposure were dermal and inhalation, although this fact is not clearly indicated in the report. Therefore, it is not known whether cataracts were a systemic or a local effect. The opacities were detectable only on the periphery of the lens and appeared continuous or discontinuous. The opacities of the lens were bilateral and symmetrical and did not affect visual fields or visual acuity. The workroom 2,4,6-trinitrotoluene air concentration was about 0.3 mg/m^3 with a range of 0.14-0.58 mg/m^3 (Harkonen et al. 1983). There was no control population in the study. The formation of cataracts did

not progress further after exposure to 2,4,6-trinitrotoluene was terminated; however, the cataracts were not reversible. The mechanism of 2,4,6-trinitrotoluene cataract formation is not understood, but the authors raised the possibility that oxidative damage may play a role since cellular defense mechanisms protective against oxidative damage (i.e., glucose-6-phosphate dehydrogenase and glutathione concentration) may be deficient after exposure to 2,4,6-trinitrotoluene (Harkonen et al. 1983).

No studies were located regarding ocular effects in animals after dermal exposure to 2,4,6-trinitrotoluene.

2.2.3.3 Immunological and Lymphoreticular Effects

Two ammunition plant workers developed an allergic contact dermatitis with erythema (Goh 1988) and erythematous papillar rush with edema (Goh and Rajan 1983) after intermediate exposures to 2,4,6-trinitrotoluene. Patch tests were used to identify the allergen using 5% as the most suitable concentration (Goh and Rajan 1983). Dermal reactions were observed to occur on the exposed parts of the body such as the hands and forearms. Once removed from environments containing 2,4,6-trinitrotoluene, the workers sensitivity subsided and the dermatitis disappeared.

No studies were located regarding immunological or lymphoreticular effects in animals after dermal exposure to 2,4,6-trinitrotoluene.

2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans or animals after dermal exposure to 2,4,6-trinitrotoluene.

2.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after dermal exposure to 2,4,6-trinitrotoluene.

2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to 2,4,6-trinitrotoluene.

2.2.3.7 Genotoxic Effects

Two studies involving genotoxic effects in individuals occupationally exposed to 2,4,6-trinitrotoluene suggested that in addition to inhalation exposure, dermal exposure may have occurred (Ahlborg et al. 1985, 1988a). These studies are discussed in detail in Section 2.2.1.7. No other studies were located regarding genotoxic effects in humans or animals after dermal exposure to 2,4,6-trinitrotoluene.

Other genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

In a case of chronic occupational exposure to 2,4,6-trinitrotoluene, a 61-year-old male died of hepatocellular carcinoma (Garfinkel et al. 1988). The routes of exposure were not specified in the study, and it is possible that the worker was exposed via the inhalation and/or dermal route.

No studies were located regarding cancer in animals after dermal exposure to 2,4,6-trinitrotoluene.

2.3 TOXICOKINETICS

Occupational studies indicate humans readily absorb 2,4,6-trinitrotoluene dusts via inhalation or dermal contact, but quantitative studies have not been done. Toxicokinetic data from animal studies are limited in that the fate of the radiolabelled dose of 2,4,6-trinitrotoluene was followed, and it is not possible to differentiate between parent compound and metabolites. However, studies in dogs, rabbits, mice, and rats indicate that more than 60% of the administered dose of 2,4,6-trinitrotoluene is absorbed when ingested; dermal exposure these animals results in significantly lower (16-68%) absorbance than oral exposure.

No studies of the distribution of 2,4,6-trinitrotoluene in humans were located. However, in animal studies the highest concentrations of 2,4,6-trinitrotoluene are found in the liver, skeletal muscle, blood, and fat.

Studies indicate 2,4,6-trinitrotoluene is metabolized to several identifiable intermediates in the urine of exposed workers, including the major reduction product aminodinitrotoluene (ADNT). In several studies of oral exposure of rats and other laboratory animals, 2,4,6-trinitrotoluene was rapidly metabolized to molecules too low in concentration to be identified by chromatographic analysis of urine. Trace amounts of 2,4,6-trinitrotoluene could be detected in the urine of exposed animals in isolated cases. After a single dermal exposure of 2,4,6-trinitrotoluene administered to dogs, rabbits, mice, and rats, more unchanged 2,4,6-trinitrotoluene was found in the urine than was found after oral exposure. This result indicates the route of exposure may influence the rate and extent of 2,4,6-trinitrotoluene metabolism.

No studies of the excretion of 2,4,6-trinitrotoluene in humans were located. However, studies indicate that 2,4,6-trinitrotoluene and its metabolites are primarily eliminated in the urine in laboratory animals. In most studies excretion is rapid.

The mechanisms by which 2,4,6-trinitrotoluene and its derivatives exert their toxic effects are largely unknown. A theory of the mechanism of toxicity by 2,4,6-trinitrotoluene is that the chemical and some of the metabolic intermediates of 2,4,6-trinitrotoluene generate reactive oxygen species that cause lipid peroxidation in the liver and injury of the lens resulting in cataracts.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Studies that directly measure the absorption of 2,4,6-trinitrotoluene in humans following inhalation exposure of known amounts of this chemical were not located. The amount of 2,4,6-trinitrotoluene in the urine as measured by Webster's reaction and the amount of 2-ADNT in the urine were compared in 88 factory workers working with 2,4,6-trinitrotoluene (Hassman and Hassmanova 1976). The

concentration of 2,4,6-trinitrotoluene in the air varied from 0.045 to 0.93 mg/m^3 in different parts of the plant. The results showed that there was a good correlation between the amounts of 2,4,6-trinitrotoluene and its main metabolite ADNT in the urine of exposed workers. The levels of ADNT increased rapidly during the workday and then declined within 24 hours to close to the levels at the beginning of the workday. These results suggest that 2,4,6-trinitrotoluene is rapidly absorbed and eliminated in the course of an acute inhalation exposure. The study is limited in that there was no information on the route of exposure, the length of exposure in the course of a working day, or the exposure dose.

In an attempt to simulate inhalation exposure, 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene suspended in methyl cellulose was instilled into the trachea of anesthetized, tracheotomized Sprague-Dawley rats (Army 1981d). At the same time, another group of rats was treated orally with the same dose of radiolabelled 2,4,6-trinitrotoluene. Both groups were sacrificed 4 hours later, and tissue and urine samples were collected for radioactivity analysis. The rate of absorption was faster after intratracheal instillation than after oral administration of 2,4,6-trinitrotoluene. Urinary excretion averaged 19.3% of the dose after intratracheal administration and 14.6% of the dose after oral administration. These results indicate that there are differences in the absorption rate of 2,4,6-trinitrotoluene depending on the administration route.

2.3.1.2 Oral Exposure

Discoloration of the urine is among the first indications that metabolism has occurred after 2,4,6-trinitrotoluene absorption in humans. The color of urine ranges from abnormal amber to deep red (Army 1978a).

Similar observations were made in rats and mice. Sixty minutes after a single exposure to 10,000 mg/kg/day of 2,4,6-trinitrotoluene, the urine of mice and rats becomes red in color (Dilley et al. 1982b). This is an indirect indication of 2,4,6-trinitrotoluene absorption. A more direct estimate of absorption of 2,4,6-trinitrotoluene was done in rats, mice, rabbits, and dogs after a single oral dose of 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene (Army 1981d). Twenty-four hours later, the recovery of radiolabel was measured in rats, mice, dogs, and rabbits. The largest percentage of radioactivity

was recovered from urine: 59.5%, 59%, and 61% for rats, mice, and dogs, respectively. Rabbits had a slightly higher recovery (74.3%) of radiolabelled 2,4,6-trinitrotoluene in their urine, with a proportional decrease in the radioactivity recovered from the gastrointestinal tract and feces (Army 1981d). Total recovery of the radioactivity (including feces, gastrointestinal tract, and urine) was 92.2%, 94.4%, 94.2%, and 103.6% in rats, mice, dogs, and rabbits, respectively. The red pigment in the urine was not detected in rabbits or dogs. The results of this study indicate that 2,4,6-trinitrotoluene is relatively quickly absorbed after oral administration and that a majority of the ingested compound is excreted within 24 hours.

Findings in rats, mice, and dogs support the observations made in humans. Discoloration of urine was noted in both rats and mice exposed to 34.7 and 35.7 mg/kg/day, respectively, for 13 weeks (Dilley et al. 1982b). Dogs treated with 20 mg/kg/day for 13 weeks had urine that was orange in color (Dilley et al. 1982b), while dogs receiving 8 or 32 mg/kg/day for 26 weeks had light to dark brown urine throughout the treatment period (Levine et al. 1990b). No adverse hepatic effects and no change in urine color were observed in monkeys treated with 1 mg/kg/day for 90 days (Martin and Hart 1974). The Martin and Hart (1974) study was limited in that only three monkeys per sex per group were utilized, thereby precluding statistical analyses of the data. Also, a high frequency of emesis was observed in the treated monkeys. It is believed that the species differences in urine color are due to the presence of unidentified metabolites of 2,4,6-trinitrotoluene. Species differences in 2,4,6-trinitrotoluene toxicity may be attributed to the different metabolic pathways of 2,4,6-trinitrotoluene and its metabolites. Once identified, these urine metabolites may be used as markers of 2,4,6-trinitrotoluene exposure.

2.3.1.3 Dermal Exposure

Although data are limited regarding absorption of 2,4,6-trinitrotoluene following dermal exposure in humans, it appears that it occurs rapidly (Woollen et al. 1986). 2,4,6-Trinitrotoluene absorption was assessed by measuring the urinary concentration of one of its metabolites, ADNT, in 25 exposed workers. There were wide variations between individual workers in the rate of clearance of ADNT from the body. Furthermore, when urine samples were collected from a subgroup of workers from the original group of 25, eight out of nine subjects had detectable ADNT levels in their urine even though

these workers had been away from the workplace for 17 days. This is an indication that a portion of absorbed 2,4,6-trinitrotoluene or its metabolites is slowly excreted (Woollen et al. 1986). Additionally, when five workers from the total group of 25 exposed workers were monitored more closely during two workshifts, it was shown that 2,4,6-trinitrotoluene was absorbed rapidly during the exposure period. The limitations of this study are that the dermal exposure dose was not measured and workers' were also exposed to 2,4,6-trinitrotoluene via the inhalation route.

The differences in absorption and excretion of radiolabelled 2,4,6-trinitrotoluene after dermal and oral exposures were investigated in mice, rabbits, rats, and beagle dogs (Army 1981d). Rats and mice were exposed dermally and orally (by gavage) to 50 mg/kg, and dogs and rabbits to 5 or 50 mg/kg. Twenty-four hours after exposure, animals were sacrificed and urine, gastrointestinal tract, feces, and various tissues were analyzed for radioactivity. Total recovered radioactivity was significantly lower in all species after dermal exposure as compared to after oral exposure (Army 1981d). The highest degree of dermal absorption was observed in rabbits and mice, in which 68.3% and 41.7% of the administered radiolabel was recovered, respectively. The total recovery of radiolabel in dogs and rats was much lower, 17% and 24%, respectively. These results suggest that absorption of radiolabelled 2,4,6-trinitrotoluene is species-dependent and is significantly lower after dermal rather than oral exposure.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution following inhalation exposure to 2,4,6-trinitrotoluene in humans.

In rats that received 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene by intratracheal administration, the highest tissue concentrations of radiolabel after 4 hours were found in both male and female animals in the fat (82 and 155 μg eq/g, respectively) and gastrointestinal tract (82 and 40 μg eq/g, respectively) (Army 1981d). Since the gastrointestinal tract contained considerable amounts of radioactivity, some of the rats in this experiment were bile-duct cannulated in order to collect bile and estimate the amount

of radiolabel. The results were given as a percent of the administered dose. The radioactivities recovered in bile-duct cannulated male and female rats were 20% and 15%, respectively, from the bile, and 18% and 13%, respectively, from the urine (Army 1981d). When these results were compared to excretion results following oral administration, the percent of radioactivity recovered in the urine and bile was significantly higher after intratracheal exposure in both male and female rats. The opposite was true for the amount of radioactivity recovered in the gastrointestinal tract; it was significantly higher in the orally treated rats regardless of cannulation (Army 1981d). These results indicate that the route of administration contributes to the differences in 2,4,6-trinitrotoluene distribution.

2.3.2.2 Oral Exposure

No studies were located regarding distribution following oral exposure to 2,4,6-trinitrotoluene in humans.

Twenty-four hours after administration of a single oral dose of radiolabelled 2,4,6-trinitrotoluene to rats and mice (100 mg/kg), and rabbits and dogs (5 mg/kg), the blood and different tissues were analyzed for radioactivity. The blood and liver, kidney, spleen, lungs, brain, and skeletal muscle of dogs contained a higher percentage of radioactivity than the blood and tissues of rats, mice, and rabbits (Army 1981d). Recovery of radioactivity was greatest in the liver, skeletal muscle, and blood in all four species. However, the amount of radioactivity recovered from tissues was small, ranging from <0.1% to 5.4% of the dose, because the majority of the label is excreted in urine (an average of 60% of the dose) and feces (an average of 11% of the dose) (Army 1981d). This indicates both rapid absorption and rapid distribution in different species after oral exposure to 2,4,6-trinitrotoluene. The limitations of this study are that a small number of animals was analyzed for distribution of radiolabel and that the amount of unchanged 2,4,6-trinitrotoluene was not discussed.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution following dermal exposure to 2,4,6-trinitrotoluene in humans or animals. However, the recovery of radiolabel after a single dermal application of 50 mg/kg of 2,4,6-trinitrotoluene was significantly lower in rats, mice, dogs, and rabbits than in those exposed

orally (Army 1981d). Skin and fat around the site of dermal application were not included in the final radiolabel recovery estimates. This may account for the lower radiolabel recovery obtained after the dermal exposure (Army 1981d). The recovery of radiolabel from urine, feces, gastrointestinal tract, blood, and tissue differed among the four species examined: rabbits (56.9%) > mice (41.7%) > rats (22.8) > dogs (15.9%).

2.3.3 Metabolism

2.3.3.1 Inhalation Exposure

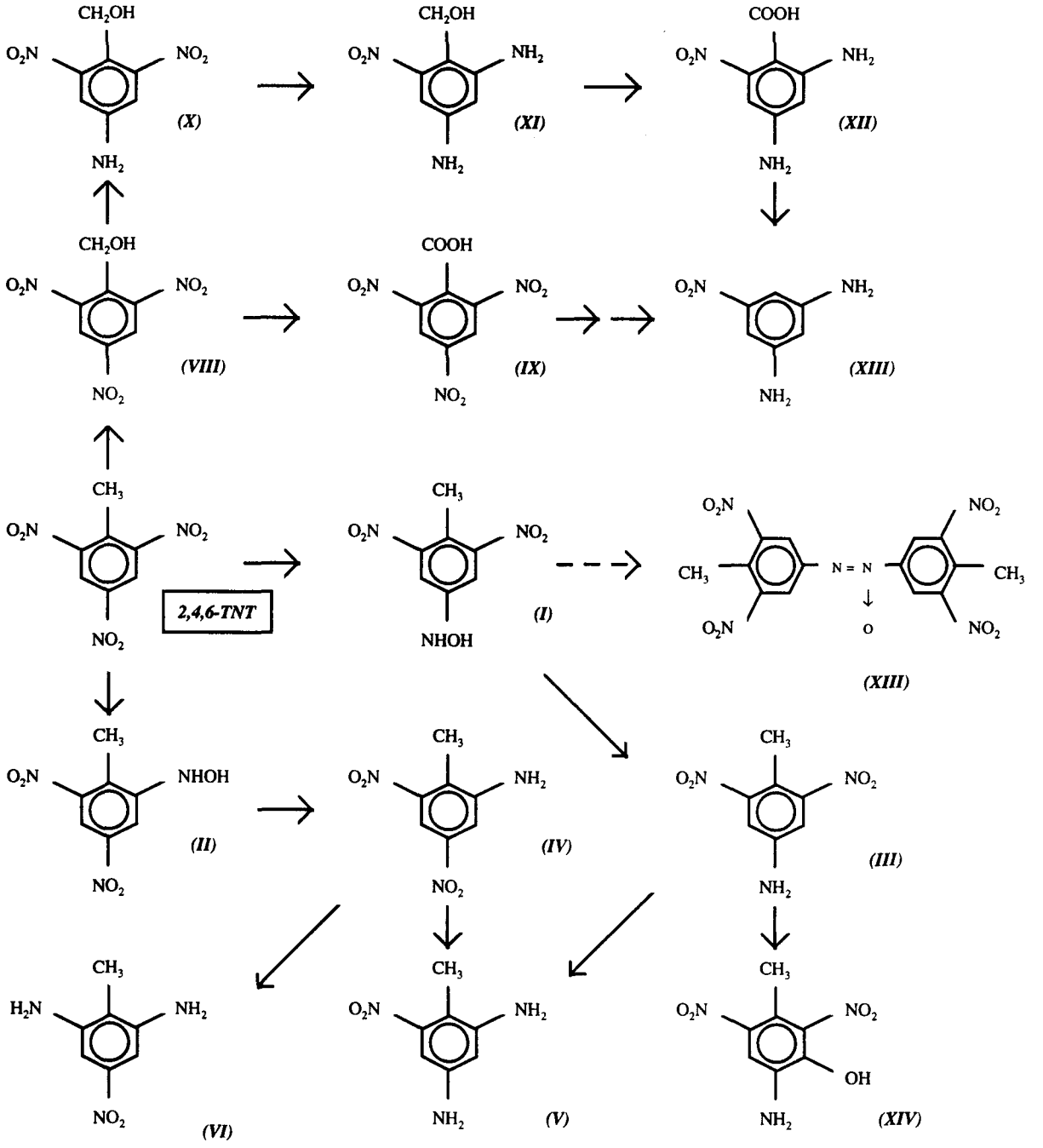
No studies were located regarding metabolism following inhalation exposure to 2,4,6-trinitrotoluene in humans or animals. However, in a retrospective study (covering a 5-year period) of 2,4,6-trinitrotoluene workers, no correlation was found between the presence in urine of one of the main 2,4,6-trinitrotoluene metabolites, ADNT, and the results of Webster's reaction, which measures urine 2,4,6-trinitrotoluene levels (Hassman and Hassmanova 1976). This result provides indirect evidence that 2,4,6-trinitrotoluene is metabolized completely and that no detectable amounts of unchanged compound are present in the urine. This study is limited in that it does not clearly define the route of exposure and does not specify the dose or the length of exposure.

2.3.3.2 Oral Exposure

No studies were located specifically addressing metabolism following oral exposure to 2,4,6-trinitrotoluene in humans.

The 2,4,6-trinitrotoluene molecule may undergo various metabolic transformations, such as oxidation of the methyl group, oxidation of the benzene ring, reduction of the three nitro groups, and conjugation (EPA 1989b). A metabolic pathway illustrating some possible transformation products of 2,4,6Mnitrotoluene is shown in Figure 2-2 (Army 1981d). The failure to detect unmetabolized 2,4,6-trinitrotoluene in the urine of humans (Hassman and Hassmanova 1976) provides indirect evidence that 2,4,6-trinitrotoluene is extensively metabolized. Trace amounts of unmetabolized 2,4,6-trinitrotoluene were found in the urine of rats, mice, rabbits, and dogs (Army 1981d). Several

Figure 2-2. Possible Biotransformation Products of 2,4,6-TNT*



*Adapted from Army 1981d

(I) 4-hydroxylamino-2,6-dinitrotoluene; (II) 2-hydroxylamino-4,6-dinitrotoluene; (III) 4-amino-2,6-dinitrotoluene; (IV) 2-amino-4,6-dinitrotoluene; (V) 4,6-diamino-2-nitrotoluene; (VI) 2,6-diamino-4-nitrotoluene; (VII) 2,6,2',6'-tetranitro-4,4'-azoxytoluene; (VIII) 2,4,6-trinitrobenzylalcohol; (IX) trinitrobenzoic acid; (X) 4-amino-2,6-dinitrobenzylalcohol; (XI) 2,4-diamino-6-nitrobenzylalcohol; (XII) 2,4-diamino-6-nitrobenzoic acid; (XIII) 5-nitro-*m*-phenylenediamine; (XIV) 4-amino-2,6-dinitro-*m*-cresol

metabolites have been identified in human urine: 4-ADNT, 2-ADNT, 2,4-diatnino-6-nitrotoluene, 4-hydroxylamino-2,6-dinitrotoluene, and amino-nitrocresol (Army 1986c; Channon et al. 1944; Lemberg and Callaghan 1945).

2,4,6-Trinitrotoluene was extensively metabolized in rats, mice, dogs, and rabbits after a single oral dose of 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene (Army 1981d). Only minute amounts of the unmetabolized 2,4,6-trinitrotoluene were found in urine. The majority of urinary metabolic products have high polarity and very low extractability in organic solvents. It was therefore difficult to identify them. The metabolic profiles of urine from the four species differed only quantitatively (Army 1981d). 4,6-Diamine, 2,6-diamine, and monoamines of 2,4,6-trinitrotoluene were the predominant metabolites detected in the urine of rats. Smaller quantities of 2- and 4-hydroxylamines and azoxytoluene were present. In contrast to rat urine, greater amounts of the monoamines and hydroxylamines and smaller quantities of polar metabolites and diamines were found in the urine of mice. The urine of dogs contained appreciable amounts of diamines and monoarnines and small amounts of the 4-hydroxylamine and 2-hydroxylamine. Substantial amounts of monoarnines, hydroxylamines, and diamines were noted in rabbit urine (Army 1981d). Treatment of urine of all species with β-glucuronidase increased the amount of extractable radioactivity, indicating that conjugation of 2,4,6-trinitrotoluene metabolites with UDP-glucuronic acid is an important route of metabolism. Urine from treated mice contained the least amount of glucuronide conjugates (Army 1981d).

2.3.3.3 Dermal Exposure

No studies were located regarding metabolism following dermal exposure to 2,4,6-trinitrotoluene in humans.

The differences in metabolic profiles from the urine of mice, rats, dogs, and rabbits after a single dermal dose of 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene were only quantitative (Army 1981d). There was an increased amount of unchanged 2,4,6-trinitrotoluene in urine after a single dermal exposure which was not found after a single oral exposure (Army 1981d). This suggests that the exposure route plays a role in the extent of 2,4,6-trinitrotoluene biotransformation.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

4-ADNT, which is considered to be a major metabolite of 2,4,6-trinitrotoluene, was shown to be present in the urine of munitions workers exposed to 0.045-0.93 mg/m^3 of 2,4,6-trinitrotoluene by both the Webster reaction and by a polarographic technique (Hassman and Hassmanova 1976). Similar findings were made using a sensitive gas chromatographic method (Almog et al. 1983). No unchanged 2,4,6trinitrotoluene was detected in this study. However, no detail was provided regarding the exposure dose or route.

Recovery of radiolabel from urine of Sprague-Dawley rats 4 hours after intratracheal instillation of 50 mg/kg of radiolabelled 2,4,6-trinitrotoluene was similar to the recovery after oral exposure. The amount of radiolabel in the urine, expressed as a percentage of the dose, was 19% and 13% in male and female animals, respectively (Army 1981d).

2.3.4.2 Oral Exposure

No studies were located regarding excretion following oral exposure to 2,4,6-trinitrotoluene in humans.

The results from animal studies indicate that urine is the major excretion route after a single oral dose of radioactive 2,4,6-trinitrotoluene. The excretion of radioactive label was studied in Sprague-Dawley rats after a single oral dose of 100 mg/kg (Army 1981d). In the course of 24 hours after exposure, 53-65% of the radioactivity was recovered from the urine; 2-8% from the feces; and 30-34% from the gastrointestinal tract and its contents. Similar results on the recovery of radiolabel in urine were obtained after oral exposure of albino CD1 mice (100 mg/kg), rabbits (5 mg/kg), and dogs (50 mg/kg) to radioactive 2,4,6-trinitrotoluene (Army 1981d). These results were confirmed when a 24-hour recovery of radiolabelled 2,4,6-trinitrotoluene was evaluated in rats, mice, dogs, and rabbits after a single oral exposure of 50 mg/kg (Army 1981d). The percentages of radiolabel in urine were 59.5%, 59%, and 61% for rats, mice, and dogs, respectively. The highest amount of radiolabel was recovered from the urine of rabbits, 74.3%. The amounts of label from feces were 11%, 24%, 5%, and 22% for

rats, mice, rabbits, and dogs, respectively. Although the number of animals in these studies was relatively small, the results indicate that there are species differences regarding excretion after acute oral exposure to 2,4,6-trinitrotoluene. The urine of rats and mice in these studies was bright red in color, indicating formation of some species-specific, unknown metabolite(s); no such color was observed in dogs and rabbits (Army 1981d).

2.3.4.3 Dermal Exposure

No studies were located regarding excretion following dermal exposure to 2,4,6-trinitrotoluene in humans.

Total recovery of radioactive label (from blood, liver, kidneys, lungs, spleen, brain, muscle, gastrointestinal tract, feces, and urine) after a single dermal exposure of 50 mg/kg of radiolabel 2,4,6-trinitrotoluene was evaluated in rats, mice, dogs, and rabbits and compared to total recovery after oral exposure (Army 198 Id). In all species, the total recovery of the radiolabel was significantly lower after dermal exposure as compared to oral exposure. Total radioactivity recovered after dermal exposure was 16%, 23%, 42%, and 57% in dogs, rats, mice, and rabbits, respectively. For comparison, the recovery after oral exposure was 92%, 94%, 94%, and 104% in rats, dogs, mice, and rabbits (Army 1981d). These results indicate that the total recovery of the label after a single dermal exposure to 2,4,6-trinitrotoluene varies depending on the species. Recovery is also affected by the exposure route; recovery after oral exposure is significantly higher than after dermal exposure (Army 1981d).

2.3.5 Mechanisms of Action

Although the mechanisms by which 2,4,6-trinitrotoluene and its derivatives exert their toxic effects on organ systems (including the blood, liver, and eye lens) are largely unknown, a general theory has been developed to explain the toxicity of 2,4,6-trinitrotoluene.

As discussed in Section 2.3.1, in limited studies of humans and animals it appears 2,4,6-trinitrotoluene is readily absorbed by inhalation, ingestion, or dermal routes of exposure. Also, it has been

demonstrated that 2,4,6-trinitrotoluene is lipid soluble; tracer studies have shown accumulation in subcutaneous fat, and it would be expected to be present in lipids in the liver and marrow. A theory of the mechanism of toxicity by 2,4,6-trinitrotoluene is that the parent compound and some metabolic intermediates are active oxygen generators (Kong et a1.1989) and are involved in lipid peroxidation in the liver and in oxygenation of the lens to form cataracts (Liu et al. 1992; Savolainen et al. 1985). In addition, it is postulated that in the rat 2,4,6-trinitrotoluene undergoes rapid reduction to hydroxylamine and that this intermediate can be bioactivated to bind sulfhydryl proteins (Liu et al. 1992). This bioactivation is postulated to occur in the liver or in the blood via different pathways. In the liver, bioactivation of hydroxylamine is postulated to involve oxidation by NADPH-dependent hepatic microsomal enzymes. In the blood, bioactivation of hydroxylamine is postulated to involve a reaction with oxyhemoglobin (Liu et al. 1992).

2.4 RELEVANCE TO PUBLIC HEALTH

The general public is not likely to be exposed to 2,4,6-trinitrotoluene. However, there is a possibility that populations will be exposed in the vicinity of munitions fabrication plants, demilitarization facilities, and particularly at incinerator facilities and former and current open-bum and opendetonation facilities. Occupational or accidental exposure to 2,4,6-trinitrotoluene may occur by the oral, inhalation, or dermal routes.

Oral exposure to 2,4,6-trinitrotoluene in aquatic environments in the vicinity of ammunition plants is not likely because 2,4,6-trinitrotoluene stays unchanged for only a short period because of photolysis (half-life of less than 24 hours) and biological degradation (half-life of less than 65 days). However, if there is 2,4,6-trinitrotoluene-contaminated soil in the vicinity of a munitions plant, oral exposure through terrestrial food products (especially homegrown produce or locally grazed animals) cannot be completely ignored as a possible exposure pathway. Dermal exposure through contaminated soil is more likely since the degradation of 2,4,6-trinitrotoluene in soil is less effective than in water (Army 1986c). Volatilization of 2,4,6-trinitrotoluene from surface water is negligible, making inhalation exposure unlikely.

2. HEALTH EFFECTS

Information on the effects that occur in humans after exposure to 2,4,6-trinitrotoluene comes from case reports of accidental intoxication and from studies of occupationally exposed workers in the manufacture of high explosives. Because of improvements in the work environment, numerous adverse health effects caused by exposure to 2,4,6-trinitrotoluene, such as anemia, liver function abnormalities, respiratory complications, and possibly aplastic anemia, have been greatly reduced.

Historically, adverse effects on the liver have caused the greatest number of deaths among munitions workers; 475 deaths were reported in the United States during World War I (McConnell and Flinn 1946). Cases of aplastic anemia, which is usually fatal, were also reported during that time. As stated in the previous paragraph, these adverse effects have been almost eliminated with the introduction of more effective protection measures for workers handling 2,4,6-trinitrotoluene.

The major effects observed in animals after intermediate or chronic exposures to 2,4,6-trinitrotoluene are reduced number of red blood cells, reduced hemoglobin and hematocrit, anemia, testicular damage, hepatomegaly, and splenomegaly.

Inhalation

No MRLs were derived for acute, intermediate, or chronic exposure by the inhalation route.

Oral

An MRL of 0.0005 mg/kg/day has been derived for intermediate oral exposure to 2,4,6-trinitrotoluene. This MRL is based on the occurrence of dose-related liver effects (cloudy swelling and hepatocytomegaly) noted in dogs administered 0.5 mg/kg/day by capsule for 6 months (Levine et al. 1990b). More severe liver injury (hemosiderosis in Kupffer’s cells and hepatic cirrhosis) was observed at high doses, supporting the sensitivity of the selected end point. In addition, liver toxicity (jaundice, elevated serum and urine bilirubin levels, hepatocellular hyperplasia, cloudy swelling, focal necrosis, changes in the levels of serum triglycerides, and increased serum cholesterol levels) have been reported in animals orally

exposed to 2,4,6-trinitrotoluene for intermediate or chronic durations (Army 1984a; Dilley et al. 1982b; Levine et al. 1984).

Insufficient information was available to calculate an acute-duration oral MRL for neurological effects. Additional studies in acute oral exposures to 2,4,6-trinitrotoluene are needed to determine the threshold level for neurological effects. Chronic-duration exposure to the intermediate-duration oral MRL of 0.0005 mg/kg/day, which is the same value as the EPA's chronic oral Reference Dose (RfD), would not be anticipated to cause adverse health effects.

Death. In the United States, 475 deaths were reported among munitions workers during World War I (McConnell and Flinn 1946). One death from hepatocellular carcinoma was reported in a case of chronic occupational exposure to 2,4,6-trinitrotoluene (Garfmkel et al. 1988). It is not clear, however, if 2,4,6-trinitrotoluene played a role in the carcinogenic process. Death has been observed in rats, mice, and dogs (Dilley et al. 1982b; Levine et al. 1990b) after oral exposure to sufficient amounts of 2,4,6-trinitrotoluene. Reported oral LD_{50} values are 1,010 and 1,320 mg/kg/day for male rats, and 820 and 795 mg/kg/day for female rats (Army 1978b; Dilley et al. 1982b). Acute oral LD_{50} values in male and female mice are 1,012 and 660 mgkglday, respectively (Army 1978b; Dilley et al. 1982b). 2,4,6-Trinitrotoluene was lethal in beagle dogs receiving 32 mg/kg/day orally for 26 weeks (Levine et al. 1990b).

It is extremely unlikely that sufficient levels of 2,4,6-trinitrotoluene could be ingested acutely by persons living in the vicinity of an ammunition plant to cause death. Furthermore, the low levels of 2,4,6-trinitrotoluene that are likely to be present in the soil near the plants are substantially below the levels that are necessary to cause death.

Systemic Effects

Respiratory Effects. Extremely limited information was located regarding respiratory effects in humans after exposure to 2,4,6-trinitrotoluene. One study of occupational exposure (Morton et al. 1976) reported several cases of respiratory difficulties in workers exposed to 2,4,6-trinitrotoluene levels

in the air that were above the TLV of 0.5 mg/m^3 (ACGIH 1993). However, there are several limitations to this study. The report does not state the exact air concentration of 2,4,6-trinitrotoluene, the exposure duration, or the number of exposed workers reporting difficulties. No details are given about the nature of the reported difficulties. No studies in animals were located that described potential respiratory effects of 2,4,6-trinitrotoluene. Therefore, insufficient evidence exists to assess the relevance of these findings to public health.

Cardiovasclilar Effects. No studies were located regarding cardiovascular effects in humans. Intermediate oral exposure to doses as high as 32 mg/kg/day of 2,4,6-trinitrotoluene for 26 weeks did not cause any changes in electrocardiogram or heart rates in beagle dogs (Levine et al. 1990b). The available information is not sufficient to evaluate the effects of 2,4,6-trinitrotoluene on populations living close to ammunition plants.

Gastrointestinal Effects. There are no studies on gastrointestinal effects in humans after exposure to 2,4,6-trinitrotoluene. However, two studies in dogs reported adverse gastrointestinal effects following intermediate oral exposure to 2,4,6-trinitrotoluene. Dogs receiving 20 mg/kg/day for 13 weeks had mucoid stools and diarrhea (Dilley et al. 1982b), while inflammation of a part of the small intestine was observed in beagle dogs fed 0.5, 2, 8, and 32 mg/kg/day of 2,4,6-trinitrotoluene for 25 weeks (Levine et al. 1990b). The inflammation was dose-dependent and was more pronounced in dogs receiving the highest dose. Based on the available information, it is possible, although unlikely, that oral exposure to 2,4,6-trinitrotofuene may cause some adverse gastrointestinal effects, but it is not known if such effects would occur after dermal exposure in the vicinity of an ammunition plant or a demilitarization facility. Although dermal exposure has been indicated as the most likely route of exposure in occupational situations, limited exposure to 2,4,6-trinitrotoluene may result from ingestion of produce contaminated by deposition from fugitive particles or resuspension of contaminated soil, or from ingestion of animal products from animals that graze in the vicinity of an ammunition or demilitarization facility (Army 1986d).

Hematological Effects. Fatal cases of aplastic anemia among workers engaged in the production of explosives have not been reported in the recent literature, although they occurred in England

(Hathaway 1985) and other countries involved in World War I. The incidence of adverse health effects of 2,4,6-trinitrotoluene including aplastic anemia have decreased dramatically because of improvements in protective measures in munitions factories.

Dose-related reductions in hemoglobin and hematocrit and a 50% increase in reticulocyte counts were noted in 626 workers exposed to 2,4,6-trinitrotoluene air levels ranging from <0.1 to 1.49 mg/m^3 (Army 1976). The duration of exposure was not specified. In another study, no abnormal values for hemoglobin were found in 43 workers employed in the manufacture of 2,4,6-trinitrotoluene who were monitored for 5 months (Morton et al. 1976).

Acute hemolytic disease was described in three ammunition plant workers (Djerassi and Vitany 1975) who were also glucose-6-phosphate dehydrogenase (GGPD) deficient. This study is limited in that the exposure route is not specified, so it is not clear how relevant the finding is for general public health.

Anemia (consisting of reduced number of red blood cells and reduced hemoglobin and hematocrit) is one of the major signs of 2,4,6-trinitrotoluene toxicity. These adverse effects were observed in rats (Dilley et al. 1982b; Jiang et al. 1991; Levine et al. 1984, 1990a), mice (Dilley et al. 1982b), and dogs (Dilley et al. 1982b; Levine et al. 1990b) after intermediate oral exposure to 2,4,6-trinitrotoluene. Similar observations were made in Fischer-344 rats fed 10 or 50 mg/kg/day for 24 months (Army 1984a). In this chronic exposure study, male rats were somewhat more sensitive than female rats to the toxic effects of 2,4,6-trinitrotoluene. Reticulocytosis was present as a compensatory response to the anemic state in all animals. Methemoglobinemia was noted in rats fed 300 mg/kg/day for 13 weeks (Levine et al. 1984), in rats fed 10 or 50 mg/kg/day for 24 months (Army 1984a), and in dogs fed 32 mg/kg/day for 6 months (Levine et al. 1990b).

Bone marrow fibrosis and leukocytosis were present in rats orally exposed to 2,4,6-trinitrotoluene for 24 months or 13 weeks; these animals were also anemic (Army 1984a; Dilley et al. 1982b).

Dogs and rats had increased platelet levels after exposure for 6 and 24 months, respectively (Army 1984a; Levine 1990b). The significance of this finding was not discussed.

Based on this information, it seems unlikely that sufficient amounts of 2,4,6-trinitrotoluene would be present near ammunition plants to cause adverse hematological effects in the population living in the vicinity. Individuals who are G6PD deficient may need to be evaluated as a potentially susceptible population.

Hepatic Effects. Toxic hepatitis has been the principal manifestation of 2,4,6-trinitrotoluene toxicity in humans, and many cases recorded during World War I were fatal (Army 1987a).

Reports on adverse hepatic effects of 2,4,6-trinitrotoluene in humans have been located. Increases in hepatic enzymes (SGOT and LDH) were noted in ammunition plant workers exposed when air levels of 2,4,6-trinitrotoluene rose from 0.3 to 0.8 mg/m^3 (Morton et al. 1976). The duration of the study was 5 months. In another study, no significant differences in liver function (LDH, bilirubin, alkaline phosphatase, SGOT and SGPT) were noted in a cross-sectional study on 626 munitions workers exposed to 0.5 mg/m^3 of 2,4,6-trinitrotoluene (Army 1976).

In a retrospective study spanning 20 years, liver cell irritation (measured with the MacLagen thymol turbidity test) was present in 40 munitions workers (Goodwin 1972). The report did not specify the exposure dose or route. Exposure of animals to moderate-to-high levels (0.5-200 mg/kg/day) of 2,4,6-trinitrotoluene over intermediate-to-chronic periods has been reported to cause adverse effects such as jaundice, elevated serum and urine bilirubin levels, hyperplasia, cloudy swelling, focal necrosis and cirrhosis of the liver, changes in the levels of serum triglycerides, and increased serum cholesterol levels (Army 1984a; Dilley et al. 1982b; Levine et al. 1984, 1990b). It is not known if chronic exposure to the levels of 2,4,6-trinitrotoluene described above would cause similar adverse hepatic effects in exposed humans.

These degenerative effects are distinct from the adaptive changes observed in livers of a number of nonhuman species in response to exposure to 2,4,6-trinitrotoluene. The most common adaptive change observed in several animal species during intermediate exposure to 2,4,6-trinitrotoluene was an increase in liver weight and/or size (Dilley et al. 1982b; Jiang et al. 1991; Levine et al. 1984). It is not known if these effects would occur in humans.

Renal Effects. No studies were located regarding renal effects in humans after exposure to 2,4,6-trinitrotoluene. However, studies have shown that discoloration of the urine is among the first indications of 2,4,6-trinitrotoluene exposure in humans and is due to the presence of 2,4,6-trinitrotoluene metabolites. The color of urine ranges from abnormal amber to deep red (Army 1978a).

Discoloration of the urine from the presence of 2,4,6-trinitrotoluene metabolites also occurs in rats, mice, and dogs. Sixty minutes after acute exposure to 10,000 mg/kg/day of 2,4,6-trinitrotoluene, the urine in mice and rats became red in color because of the presence of 2,4,6-trinitrotoluene metabolites (Dilley et al. 1982b). The same effect was observed in rats and dogs treated with higher doses and for a longer period (Dilley et al. 1982b). Increased filtration rate was present in rats chronically exposed to 10 and 50 mg/kg/day of 2,4,6-trinitrotoluene (Army 1984a).

In rats treated with higher doses of 2,4,6-trinitrotoluene for 13 weeks (Levine et al. 1984; 1990b) or 24 months (Army 1984a), histopathological analysis revealed the accumulation of yellowish-brown pigment in the renal cortex and in the epithelial cells of proximal convoluted tubules. It is therefore possible that persons exposed to extremely high levels of 2,4,6-trinitrotoluene may be at increased risk of renal toxicity.

Dermal Effects. Exposure to 2,4,6-trinitrotoluene can cause dermatitis in workers handling the compound (Morton et al. 1976). Two incidences of allergic contact dermatitis were reported in two ammunition plant workers after intermediate-duration exposure to 2,4,6-trinitrotoluene (Goh 1988; Goh and Rajan 1983). These findings indicate that prolonged exposure to relatively low levels of 2,4,6-trinitrotoluene may cause an allergic reaction manifested by dermatitis appearing in the areas of contact with the chemical.

Ocular Effects. The appearance of irreversible cataracts is believed to be specific to 2,4,6-trinitrotoluene exposure. It is often associated with chronic exposures to relatively low levels of 2,4,6-trinitrotoluene (Hathaway 1985). Equatorial lens opacities/cataracts were reported in 6 out of 12 workers exposed to 2,4,6-trinitrotoluene for an average of 6.8 years (Harkonen et al. 1983). The average

concentration of 2,4,6-trinitrotoluene in the air was 0.3 mg/m^3, which is well below the threshold limit of 0.5 mg/m^3 (ACGIH 1993). It is therefore possible that chronic exposure to low levels of 2,4,6-trinitrotoluene in the vicinity of ammunition plants may have adverse ocular effects.

Immunological and Lymphoreticular Effects. An increase in the number of mononuclear leukocytes was found in reviewing blood cell counts of 105 individuals exposed to 2,4,6-trinitrotoluene (Army 1978a). This increase precedes any other symptom, remains positive for 2-3 months, and could be helpful in differential diagnosis. Also increased were lymphocyte numbers in nine cases of fatal 2,4,6-trinitrotoluene poisoning (Army 1974). Since the doses necessary to produce these effects were not established, the possibility that susceptible persons living in the vicinity of ammunition plants may be exposed to sufficient amounts of 2,4,6-trinitrotoluene to trigger such immunological effects cannot be excluded.

Exposure to 2,4,6-trinitrotoluene can cause dermatitis in workers handling the compound (Morton et al. 1976). Two incidences of allergic contact dermatitis were reported in two ammunition plant workers after intermediate exposure to 2,4,6-trinitrotoluene (Goh 1988; Goh and Rajan 1983). These findings indicate that prolonged exposure to relatively low levels of 2,4,6-trinitrotoluene may cause an allergic reaction manifested by dermatitis appearing in the areas of contact with the chemical.

Increased spleen weight was the most common effect seen in several nonhuman species after intermediate exposure to medium-to-high doses of 2,4,6-trinitrotoluene (Dilley et al. 1982b; Levine et al. 1984, 1990a). Other changes, such as splenic congestion and hemosiderosis, reduced lymphocyte counts, increased lymphocyte counts, and increased globulin levels, were also noted. Thus, persons exposed to sufficiently high levels of 2,4,6-trinitrotoluene near ammunition plants may be at risk of developing immune system or lymphoreticular effects including splenomegaly with splenic congestion and hemosiderosis, lymphocytosis due to reduced lymphocyte counts, and higher globulin levels.

Neurological Effects. Only minor neurological effects, such as altered taste, were noted in humans after an inhalation exposure of 0.3 mg/m^3 of 2,4,6-trinitrotoluene (Morton et al. 1976). On the basis of this limited information, it is difficult to speculate on possible adverse neurological effects that may occur in 2,4,6-trinitrotoluene-exposed people living in the vicinity of ammunition plants.

Rats showed no signs of neurotoxicity after acute exposure to 182 mgtkglday (Short and Lee 1980). However, when fed an extremely high dose of 10,000 mg/kg/day, both rats and mice showed signs of inactivity; some developed convulsions and died (Dilley et al. 1982b). Similar observations were made in the intermediate-duration studies in dogs, rats, and monkeys fed low doses of 2,4,6-trinitrotoluene (Dilley et al. 1982b; Martin and Hart 1974). When higher doses were used (32 mg/kg/day for 26 weeks), dogs became ataxic (Levine et al. 1990b). In rats exposed to 300 mg/kg/day of 2,4,6-trinitrotoluene for 13 weeks, brain lesions (consisting of focal vacuolation and malacia of the white tracts of the cerebellar folia) were seen in histopathological analysis (Levine et al. 1984). In contrast, no significant signs of neurotoxicity were seen in rats treated with up to 50 mg/kg/day for 24 months (Army 1984a). Since these results indicate species differences after acute, intermediate, and chronic exposures, it is difficult to estimate potential neurotoxic effects for humans living close to ammunition plants.

Reproductive Effects. A preliminary case control study of workers in two 2,4,6-trinitrotoluene plants in China indicates exposure to 2,4,6-trinitrotoluene may have adverse effects on several indicators of male reproductive status (Li et al. 1993). Workers exposed to 2,4,6-trinitrotoluene had significantly lower semen volumes and a smaller percentage of motile spermatozoa as well as a significantly higher incidence of sperm malformation than the control group. However, exposure concentrations and route of exposure are not known. Possible important variables which are not discussed include exposure to other chemicals and heat in the workplace.

Serious reproductive effects, such as testicular atrophy and atrophic seminiferous tubules, were observed in rats treated with high doses of 2,4,6-trinitrotoluene for 13 weeks (Dilley et al. 1982b; Levine et al. 1984, 1990a). These changes were not reversible after a 4-week recovery period. Based

on this limited information, adverse reproductive effects after exposure of males to sufficiently high concentrations of 2,4,6-trinitrotoluene cannot be excluded.

Developmental Effects. No studies were located regarding developmental effects in humans or animals following exposure to 2,4,6-trinitrotoluene by any exposure route. It is therefore not possible to predict the potential developmental toxicity of 2,4,6-trinitrotoluene at hazardous waste sites or near ammunition plants.

Genotoxic Effects. No studies were found that directly assess the potential of 2,4,6-trinitrotoluene to induce genotoxic effects in humans. However, there is convincing evidence that the urine of individuals occupationally exposed to 2,4,6-trinitrotoluene contains mutagenic components (Ahlborg et al. 1985, 1988a). The primary metabolite of 2,4,6-trinitrotoluene appears to be 4-aminodinitrotoluene (6ADNT); major intermediate forms, including 4-ADNT, are weakly mutagenic in bacteria (Spanggord et a1.1982b). By contrast, 2,4,6-trinitrotoluene is a confirmed mutagen in bacterial and mammalian cells *in vitro* and;2,4,6-trinitrotoluene-induced mutagenesis is either markedly diminished or abolished by the inclusion of exogenous metabolic activation into these test systems (see discussion of *in vitro* results below).

The detection of unmetabolized 2,4,6-trinitrotoluene in the urine of exposed workers exhibiting a high level of mutagenic activity (Ahlborg et al. 1985) tends to support the assumption that the parent compound rather than its derivatives was responsible for the observed response. However, a more detailed follow-up study found no correlation between mutagenicity and 2,4,6-trinitrotoluene concentration in urine (Ahlborg et al. 1988a). It is nevertheless possible that these conflicting results could be resolved if more appropriate concentration procedures improved the detection of 2,4,6-trinitrotoluene. This would elucidate the possible connection between mutagenesis and the concentrations of 2,4,6-trinitrotoluene and/or its metabolites in the urine of exposed workers.

Only one *in vitro* study employing a human cell line (WI-38 human fibroblasts) was found in the existing literature (Army 1978~). In this study, target cells were exposed to 2,4,6-trinitrotoluene doses ranging from 2 to 2,000 μg/mL without S9 activation and from 6 to 6,000 μg/mL in the presence of an

uninduced mouse liver homogenate. Precipitation of the test material occurred at ≥200 μg/mL (without S9) and at 375 μg/mL (in the presence of S9). At nonactivated levels of 500 and 1,000 μg/mL, a significant ($p<0.05$) increase in UDS was obscured by discoloration of the samples; however, significant ($p<0.05$) effects were also obtained at 250 μg/mL. No discoloration of the test samples occurred in the S9-activated phase of testing, and no evidence of a genotoxic response was uncovered. Although a definitive conclusion could not be reached because of compound interference with the nonactivated assay results, the data do not suggest that 2,4,6-trinitrotoluene was genotoxic in this human cell line. Similarly, the lack of an effect in the presence of auxiliary metabolic activation is consistent with other *in vitro* assay results.

The single *in vivo* animal study assessing potential adverse effect on the chromosome structure of somatic cells following oral administration of 2,4,6-trinitrotoluene was negative but compromised because neither a toxic effect in the rats nor a cytotoxic effect on the target organ (i.e., bone marrow cells) was demonstrated (Army 1978c). However, the results of a well-conducted mouse micronucleus assay, which evaluated 2,4,6-trinitrotoluene at a level (80 mg/kg) that approximated 80% of the maximum tolerated dose, provided no indication of a clastogenesis (Ashby et al. 1985). For this study, groups of five male CBA x Balb C mice received single intraperitoneal injections of 40 or 80 mg/kg 2,4,6-trinitrotoluene; animals were sacrificed 24, 48, and 72 hours post-treatment, and bone marrow cells were examined for the presence of micronucleated polychromatic erythrocytes (MPEs). Results indicated that there were no significant increases in the frequency of MPEs in bone marrow cells sampled over the entire hematopoietic cycle. Similarly, the *in vivo* / *in vitro* rat liver UDS assay performed by the same investigators was negative.

As the above discussion indicates, deleterious genetic events resulting from exposure to 2,4,6-trinitrotoluene have not been extensively investigated in either humans or animals. Since the only available studies in humans were from occupational settings, inhalation must be considered an important pathway of 2,4,6-trinitrotoluene exposure. However, quantifiable dermal absorption indicates that 2,4,6-trinitrotoluene is absorbed through the skin. It also suggests that dermal absorption plays an important role in 2,4,6-trinitrotoluene uptake, which may be more important than uptake through inhalation (Ahlborg et al. 1988a). The identification of the agent(s) responsible for the

mutagenic activity observed in the urine of workers exposed to 2,4,6-trinitrotoluene is of considerable importance, because human metabolism of 2,4,6-trinitrotoluene has not been fully characterized. It is possible that the parent compound, a known mutagen, and its metabolites, which are also mutagens in *S. typhimurium* TA98, are contributors to 2,4,6-trinitrotoluene-induced mutagenesis (Spanggord et al. 1982b).

Although only three *in vivo* studies were found, the overall results provided no evidence that 2,4,6-trinitrotoluene is genotoxic in whole animals. This assumption is supported by the results of the *in vitro* UDS assay with human cells indicating that 2,4,6-trinitrotoluene-induced UDS was abolished by the inclusion of exogenous metabolic activation (Army 1978c). Similar results, as discussed below, were obtained in other test systems using both cultured bacterial and mammalian cells.

The implications of both the whole animal and *in vitro* human cell assay findings are highly relevant to human health. If 2,4,6-trinitrotoluene can be reduced to nonmutagenic metabolic products, the potential health hazard to humans would be greatly reduced. Refer to Tables 2-2 and 2-3 for a further summary of these studies.

In contrast to the absence of genotoxicity in animal studies, numerous investigators (Ahlborg et al. 1985, 1988a; Army 1978a, 1978c, 1979b, 1980d; Kaplan and Kaplan 1982c; Pearson et al. 1979; Spanggord et al. 1982b; Whong and Edwards 1984; Won et al. 1976) have demonstrated that 2,4,6-trinitrotoluene is a microbial mutagen. There is good agreement that 2,4,6-trinitrotoluene primarily causes frameshift mutations in *S. typhimurium* TA1537, TA1538, and TA98 and that the mutagenic response is not dependent on auxiliary metabolic activation nor substantially influenced by microbial nitroreductase activity. Data also exist showing that 2,4,6-trinitrotoluene is mutagenic in *S. typhimurium* strains TA1535 and TA100, which detect agents that cause base-pair substitution mutations (Army 1978c, 1979b, 1980d; Whong and Edwards 1984). The weight of evidence, however, is consistent with a frameshift mutagen; appreciably higher levels of 2,4,6-trinitrotoluene (≥30 μg/plate) were required to achieve positive responses in TA1535 and TA100 as compared to the reactivity (≥2 μg/plate) of 2,4,6-trinitrotoluene with strains TA1538 and/or TA98. Metabolites of 2,4,6-trinitrotoluene have not been shown as consistently to be mutagens in *S. typhimurium.* While the

TABLE 2-2. Genotoxicity of 2,4,6-Trinitrotoluene *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium (TA1535, TA1537, TA1538, TA98, TA100NR)	Gene mutations	–[a]	+[b]	Whong and Edwards 1984
S. typhimurium (TA1523, TA1537, TA1538, TA98, TA100NR3)	Gene mutations	+[c]	+[b]	Spanggord et al. 1982b
S. typhimurium (TA1538, TA98)	Gene mutations	No data	+	Kaplan and Kaplan 1982c
S. typhimurium (TA1535, TA1537, TA1538, TA98, TA100)	Gene mutations	+	+	Pearson et al. 1979
S. typhimurium (TA1535, TA1537, TA1538, TA98, TA100)	Gene mutations	+[d]	+[d]	Army 1978a, 1980c
S. typhimurium (TA1535, TA1537, TA1538, TA98, TA100)	Gene mutations	+[d]	+[d]	Army 1978c, 1979b
S. typhimurium (TA98)	Gene mutations	No data	+[e]	Won et al. 1976
Mammalian cells:				
$TK^{+/-}$ Mouse lymphoma cells	Gene mutations	–	+	Styles and Cross 1983
Human (WI-38 fibroblasts)	Unscheduled DNA synthesis	–	+/–	Army 1978c

[a]Tested only with strain TA1538
[b]Strongest response in strains TA98, TA1538, TA1537; negative in strains TA1535, TA100NR
[c]Stronger response without S9 activation; negative with or without S9 in strains TA1535, TA100NR3
[d]Stronger response without S9 activation; response was also stronger with strains TA98, TA1538, TA1537
[e]Parent compound but not seven metabolites were positive

– = negative result; + = positive result; +/– = inconclusive result; DNA = deoxyribonucleic acid; NR = nitroreductase deficiency; TK = thymidine kinase

TABLE 2-3. Genotoxicity of 2,4,6-Trinitrotoluene *In Vivo*

Species (test system)	End point	Results	Reference
Mammalian cells:			
Rat (bone marrow)	Chromosome aberrations	–[a]	Army 1978c
Mouse (bone marrow)	Micronuclei induction	–[b]	Ashby et al. 1985
Mouse (liver)	Unscheduled DNA synthesis	–[c]	Ashby et al. 1985
Human (occupational exposure/urine)	Gene mutations in *Salmonella typhimurium* TA98 and TA98NR	+[d]	Ahlborg et al. 1988a
Human (occupational exposure/urine)	Gene mutation in *S. typhimurium* TA98, *Escherichia coli* WP_2 uvrA	+[e]	Ahlborg et al. 1985

[a]Negative after oral exposure but the study was compromised
[b]Intraperitoneal exposure
[c]Oral exposure
[d]Positive without auxiliary metabolism; response stronger in strain TA98
[e]Positive without auxiliary metabolism in strain TA98; negative in strain WP_2 uvrA with or without auxiliary metabolism

– = negative result; + = positive result; DNA = deoxyribonucleic acid; NR = nitroreductase deficiency

parent compound induced a dose-related increase in mutant colonies of *S. typhimurium* TA98 over a concentration range of 2-10 μg/plate, the seven investigated metabolites were negative (Won et al. 1976). In a study using the same assay system, one of the tested metabolites (2-amino-4,6-dinitrotoluene) was positive, while the second one (4-amino-2,6-dinitrotoluene) was only slightly positive; both needed nitroreductase to induce mutagenicity (Spanggord et al. 1982b). Similarly, in a third study the four possible mono- and diamino metabolites of 2,4,6-trinitrotoluene were all less mutagenic than the parent compound in TA98 or TA100 (Tan et al. 1992). Mutagenicity in *Salmonella* tester strains seems dependent on endogenous nitroreductase activity. Strains deficient in nitroreductase show decreased sensitivity to 2,4,6-trinitrotoluene while strains constructed with increased nitroreductase activity show increased sensitivity (Einisto et al. 1991).

2,4,6-Trinitrotoluene is also capable of causing gene mutations in mammalian cells (Styles and Cross 1983). In a well-conducted study, 2,4,6-trinitrotoluene (8-l,000 μg/mL) caused dose-dependent cytotoxicity and significant increases in mutation at the $TK^{+/-}$ locus in mouse lymphoma cells. In agreement with other *in vitro* assay findings, S9 activation was not required to demonstrate the response. 2,4,6-Trinitrotoluene was negative under conditions of exogenous metabolic activation. No studies investigating potential clastogenic effects *in vitro* were found.

Although the database for *in vitro* genetic toxicology testing with 2,4,6-trinitrotoluene is limited, a high degree of concordance exists among different assay systems. Based on the existing information, there is sufficient valid *in vitro* data to conclude that 2,4,6-trinitrotoluene is a direct-acting mutagen in bacterial and mammalian cells. There is also suggestive evidence that 2,4,6-trinitrotoluene is a directacting genotoxic agent in cultured human cells. Refer to Table 2-2 for a further summary of these results.

Cancer. One preliminary epidemiological study of German populations living in the proximity of former munitions plants suggests 2,4,6-trinitrotoluene may increase leukemia rates in exposed adult human populations (Kolb et al. 1993). However, the case numbers in this study are very small. Also the proximity of the cases of leukemia to sites where 2,4,6-trinitrotoluene was manufactured during World War II or to disposal sites is not reported nor are any environmental 2,4,6-trinitrotoluene

concentrations. Therefore, there is only slight circumstantial evidence linking the observed leukemia cases to possible 2,4,6-trinitrotoluene exposure. Furthermore, no investigation of confounding variables (e.g., benzene exposure or occupational exposure to carcinogens) has been done. Several female Fisher-344 rats developed urinary bladder carcinoma after exposure to 10 or 50 mg/kg/day of 2,4,6-trinitrotoluene for 24 months (Army 1984a). A similar study conducted in $B6C3F_1$ mice showed that a statistically significant ($p<0.01$) incidence of leukemia and/or malignant lymphoma of the spleen was present in female mice receiving 70 mg/kg/day for 24 months (Army 1984b). On the basis of this result, EPA has classified 2,4,6-trinitrotoluene as a possible human carcinogen (Group C) (EPA 1989b).

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 2,4,6-trinitrotoluene 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 2,4,6-trinitrotoluene are discussed 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 2,4,6-Trinitrotoluene

The availability of biomarkers is useful in estimating the degree of exposure in cases where the exposure is suspected or known. Identification of 2,4,6-trinitrotoluene in blood and urine is the most direct confirmation that exposure to 2,4,6-trinitrotoluene has occurred. Sensitive methods have been developed for determination of 2,4,6-trinitrotoluene and its metabolites in human blood and urine (for more information see Chapter 6).

Detection of 2,4,6-trinitrotoluene in the blood or urine is an indication of a recent dermal, oral, or inhalation exposure. However, since 2,4,6-trinitrotoluene is rapidly metabolized, it may be difficult to determine trace amounts of the unchanged compound in blood or urine. In such cases, identification of major 2,4,6-trinitrotoluene metabolites such as 4-ADNT and 2-ADNT in the urine can be used as an indication of exposure. In one case of acute, primarily dermal exposure to 2,4,6-trinitrotoluene, these two metabolites were present in the urine of exposed workers 17 days after exposure (Woolen et al. 1986). This finding indicates that they can be used as indicators of not only recent, but also past, acute exposures to 2,4,6-trinitrotoluene. Another early sign of 2,4,6-trinitrotoluene exposure is the

change of urine color that can range in humans from abnormal amber to deep red (Army 1978a). The identification of the metabolite responsible for this color change would represent a good biomarker for early detection of 2,4,6-trinitrotoluene exposure.

In the study on distribution of 2,4,6-trinitrotoluene after oral, inhalation and dermal exposure of rats, mice, rabbits and dogs it was found that 2,4,6-trinitrotoluene distributes to fat (Army 1981d). Therefore the lipid concentration of 2,4,6-trinitrotoluene could be used as a potential biomarker of exposure, provided adequate methodology is available.

No information was found on tissue levels of 2,4,6-trinitrotoluene after relatively long-term exposure to constant levels of 2,4,6-trinitrotoluene. However, since absorption, biodegradation, and excretion occur rapidly, it can be assumed that the presence of 2,4,6-trinitrotoluene metabolites may be used to identify exposure. Because of rapid conversion of 2,4,6-trinitrotoluene into its metabolites, it is also reasonable to assume that long-term exposure will not lead to a steady state of 2,4,6-trinitrotoluene levels.

2.5.2 Biomarkers Used to Characterize Effects Caused by 2,4,6-Trinitrotoluene

Prior to the use of blood and urine levels to monitor exposure to 2,4,6-trinitrotoluene (during the two World Wars, and especially during World War I), jaundice was one of the main indicators of 2,4,6-trinitrotoluene intoxication. However, jaundice is a sign of serious hepatic toxicity, that develops over a period of time, and it is not useful as an early indicator compared to other signs such as urine discoloration. Since jaundice is a latent phenomenon, many cases had fatal outcomes before the jaundice was observed. In addition, jaundice is not specific for 2,4,6-trinitrotoluene exposure and may be caused by other factors.

Decreased hemoglobin and hematocrit levels and increased reticulocyte numbers are among the first changes to occur after exposure to sufficiently high levels of 2,4,6-trinitrotoluene (Army 1976). The levels of these three blood parameters reflected in a complete blood count can be used as nonspecific biomarkers; their determination is rapid, relatively inexpensive, and useful for monitoring cohorts of persons possibly exposed to 2,4,6-trinitrotoluene.

An early reaction to 2,4,6-trinitrotoluene intoxication is an increase in mononuclear leukocytes, which seems to precede any other symptom and remains positive for 2-3 months (Army 1978a). Also found in nine fatal cases of 2,4,6-trinitrotoluene poisoning was a significant increase in lymphocyte counts (Army 1974a). The extent and significance of these findings need further elucidation since both are commonly present in a number of other pathological states.

Changes in the hepatic enzymes SGOT and LDH were noted after the levels of 2,4,6-trinitrotoluene increased from 0.3 to 0.8 mg/m^3 (Morton et al. 1976). In another study, however, no changes in liver function were seen in 626 munitions workers exposed to an average of 0.5 mg/m^3 (Army 1976). An explanation for these different findings may be that new or increased exposure to 2,4,6-trinitrotoluene causes more liver toxicity in potentially susceptible workers, while in cases of longer exposure liver cells may adapt to moderate exposure levels (Hathaway 1985). Although many other substances and diseases can cause changes in the levels of hepatic enzymes, a record of pre-exposure levels could resolve these problems and allow for the use of hepatic enzymes as effective exposure markers. It is possible that in the future a battery of tests to indicate liver disease could be used to identify the causal agent. For example, cholylglycine is a bile acid that accumulates in serum in cases of hepatic dysfunction, and interleukin-1 is indicative of inflammation. Both of these markers were found elevated in all patients with viral hepatitis, but only 37.5% or 25% were positive, respectively, in 2,4,6-trinitrotoluene-induced liver damage (Li et al. 1992).

Another potential adverse effect of 2,4,6-trinitrotoluene exposure is the formation of cataracts. It is believed to be specific to 2,4,6-trinitrotoluene and is often associated with chronic, low-level exposure (Hathaway 1985). Bilateral, symmetrical equatorial lens opacities were reported in workers exposed to 2,4,6-trinitrotoluene for an average of 6.8 years (Harkonen et al. 1983).

Two cases of contact dermatitis were reported in workers after an intermediate exposure to 2,4,6-trinitrotoluene (Goh 1988; Goh and Rajan 1983). On the basis of this information, skin patch testing could be done to detect individuals potentially hypersensitive to 2,4,6-trinitrotoluene.

2.6 INTERACTIONS WITH OTHER CHEMICALS

Limited information was located regarding the influence of other chemicals on the toxicity of 2,4,6trinitrotoluene. However, one extensive animal study evaluated the acute and intermediate effects of a mixture of 2,4,6-trinitrotoluene and 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX) on rats, mice, dogs, and rabbits (Army 1978c). The mixture of the two compounds is designated as LAP (load, assemble, and pack) mixture. The ratio of 2,4,6-trinitrotoluene and RDX in the LAP mixture in this study was 1.6:1. Acute oral toxicity of the LAP mixture was investigated in rats, mice, and rabbits. The results indicate that there was a distinct species difference regarding acute oral toxicity after exposure to the LAP mixture. The acute oral LD_{50} values indicate that rats were more susceptible to toxic effects of the LAP mixture than to 2,4,6-trinitrotoluene alone. The opposite was true for mice which were more resistant to the LAP mixture than to 2,4,6-trinitrotoluene (Army 1978c). LAP applied to the eyes of rabbits produced conjunctivitis, iritis, and/or cornea1 opacity. Intermediate oral toxicity was determined in a 90-day exposure study in rats, mice, and dogs. The results indicate that the main target organs for LAP toxicity are the same as those for 2,4,6-trinitrotoluene, namely blood and liver. Mild-to-moderate hemolytic anemia, enlarged spleens and livers, hemosiderosis of the spleen, and colored urine were common effects of intermediate exposure to LAP seen in all three species. LAP-induced testicular atrophy (dogs and rats), uterine hypoplasia (rats), and numerous neurological signs (dogs) were also observed. These observations indicate that 2,4,6-trinitrotoluene was the principal, but not the only factor, contributing to the intermediate oral toxicity of LAP; some of the observed toxicity is due to RDX (Army 1978c).

Because 2,4,6-trinitrotoluene is rapidly degraded in the environment, it is possible that it would interact with its degradation products to amplify adverse health effects. However, it is not known how the interaction of 2,4,6-trinitrotoluene with these co-contaminants affects or alters predicted health effects.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to 2,4,6-trinitrotoluene than will most persons exposed to the same level of 2,4,6-trinitrotoluene 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 that 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 that are known to be unusually susceptible to toxic effects of 2,4,6-trinitrotoluene. However, in the review of the literature one report described the occurrence of acute hemolytic disease (Djerassi and Vitany 1975) in three individuals deficient in GGPD enzyme. All three developed hemolytic crisis with similar symptoms 2-4 days after being exposed to 2,4,6-trinitrotoluene. All three also recovered and were complication free at 5- and 10-year followup. Populations that may show increased sensitivity to 2,4,6-trinitrotoluene would include very young children, who have immature hepatic detoxification systems; individuals with impaired liver function, including alcoholics (Li et al. 1991), or impaired kidney function; and those who are prone to anemia or who are anemic. Also at increased risk may be individuals with such genetic traits as G6PD deficiency, sickle cell trait, genetically induced unstable hemoglobin forms, or congenital hypercholesterolemia. Another subpopulation that may be at increased risk is comprised of individuals with a potential immune reaction to 2,4,6-trinitrotoluene.

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe the clinical practice and research concerning methods for reducing toxic effects of exposure to 2,4,6-trinitrotoluene. 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 2,4,6-trinitrotoluene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

No chemical-specific recommendations have been reported for reducing absorption following 2,4,6-trinitrotoluene exposure via any route (Haddad and Winchester 1990; HSDB 1994). General methods for reducing exposure can be found for explosives (Bronstein and Currance 1988). Other sources recommend the same treatment for overexposure to 2,4,6-trinitrotoluene as for aniline (Gosselin et al. 1984) or aromatic nitro compounds (Stutz and Ulin 1992). General procedures suggested for reducing absorption following accidental industrial exposure include moving the exposed person into fresh air, removing contaminated clothing and shoes, and flushing exposed skin or eyes with running water (HSDB 1994).

In recent years, there have been very few reported cases of overexposure via inhalation or dermal contact because simple industrial hygiene methods are used to effectively prevent contact with high concentrations of 2,4,6-trinitrotoluene in the workplace. However, during both World Wars some cases of industrial exposure have resulted in fatalities (Haddad and Winchester 1990).

Oral exposure to toxic quantities of 2,4,6-trinitrotoluene have not been reported for humans, although it is possible that some of the cases of overexposure of workers were caused in part by inadvertent ingestion via hand-to-mouth contact as well as by inhalation and dermal contact with 2,4,6-trinitrotoluene. In general, only supportive treatment has been recommended (HSDB 1994). In some cases, gastric lavage, activated charcoal, and emetics have been suggested as useful in reducing absorption of the general class of nitro compounds to which 2,4,6-trinitrotoluene belongs (Gosselin et al. 1984; Stutz and Ulin 1992). Other sources state emesis should not be used for explosives (Bronstein and Currance 1988).

2.8.2 Reducing Body Burden

No quantitative studies of human retention or elimination of 2,4,6-trinitrotoluene were located. Acute laboratory studies of animals show rapid elimination in the urine of 2,4,6-trinitrotoluene and its metabolites when it is administered orally or dermally. Historically, only a small proportion of the munitions workers during World War I and II who were exposed to high concentrations of

2,4,6-trinitrotoluene experienced hepatic disease. The only treatment was to remove the affected workers from areas where they could be exposed to 2,4,6-trinitrotoluene. Because the onset of symptoms was frequently delayed for 1 to 4 months following exposure, in some cases no symptoms appeared until days or weeks after exposure to 2,4,6-trinitrotoluene had ended (Haddad and Winchester 1990).

A more recent study of workers exposed to 2,4,6-trinitrotoluene found that a subgroup of workers showed signs of liver cell irritation. These workers were removed from areas with 2,4,6-trinitrotoluene exposure and fed diets high in protein and calories. After 3 weeks, signs of liver cell irritation disappeared, and they were returned to work requiring contact with 2,4,6-trinitrotoluene (Goodwin 1972).

In some cases of exposure to 2,4,6-trinitrotoluene, methemoglobinemia has been reported. This effect is independent of hepatic damage. Some sources suggest treatment of methemoglobinemia with methylene blue (Ellenhorn and Barceloux 1988; Gosselin et al. 1984; Stutz and Ulin 1992). However, it has been noted that methylene blue should be used with caution (Stutz and Ulin 1992) especially if there is a possibility of glucose-6-phosphate dehydrogenase (G6PD) deficiency (Ellenhorn and Barceloux 1988).

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

The mechanism of toxic action by 2,4,6-trinitrotoluene and the compounds formed by the metabolism of 2,4,6-trinitrotoluene are not known. A theory is that, at a biochemical level, 2,4,6-trinitrotoluene or its metabolites generate active oxygen species (Kong et al. 1989; Liu et al. 1992; Savolainen et al. 1985). No information was located on established therapies designed to interfere with this possible mechanism of action of 2,4,6-trinitrotoluene. Because 2,4,6-trinitrotoluene is known to cause liver damage and may decrease glutathione concentrations (Liu et al. 1992), it is possible an intervention that acts by increasing the cellular concentrations of antioxidants (especially glutathione, glutathione peroxidase, and those that are lipid soluble such as vitamin E) could reduce liver damage caused by 2,4,6-trinitrotoluene.

The impetus to search for an agent that reduces the toxicity of 2,4,6-trinitrotoluene or its metabolites is generally lacking since the use of good hygiene in the workplace has eliminated the health effects formerly detected in the United States and Western Europe. It should be noted that with industrial or military accidents involving large quantities of 2,4,6-trinitrotoluene, the most immediate critical hazard is one of explosion or fire (HSDB 1994; Stutz and Ulin 1992).

2.9 ADEQUACY OF THE DATABASE

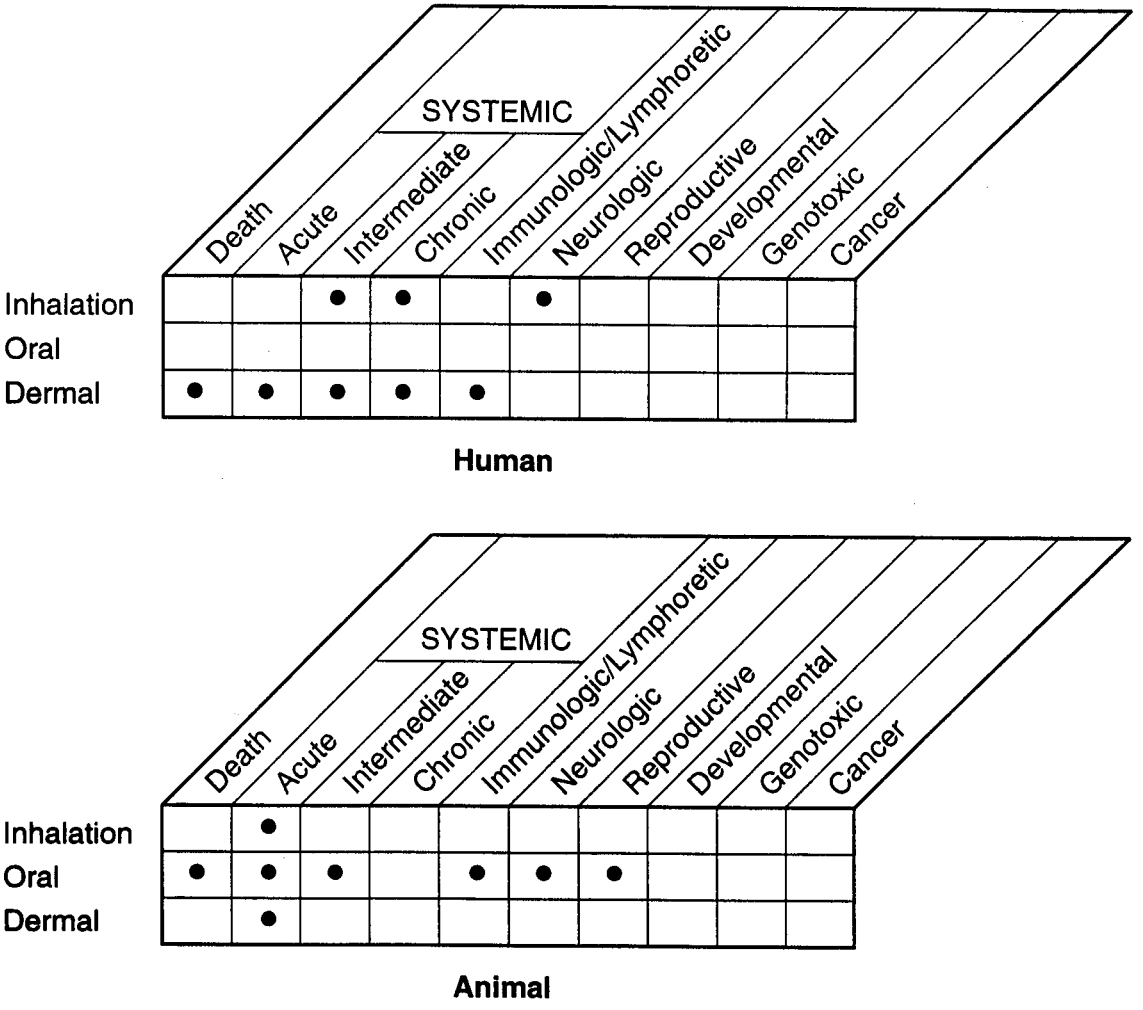
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 2,4,6-trinitrotoluene is available. Where adequate information 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 2,4,6-trinitrotoluene.

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 may be proposed.

2.9.1 Existing Information on Health Effects of 2,4,6-Trinitrotoluene

The existing data on health effects of inhalation, oral, and dermal exposure of humans to 2,4,6-trinitrotoluene are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of 2,4,6-trinitrotoluene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Missing information in this figure should not be interpreted as “data needs” information (i.e., data gaps that must necessarily be filled).

FIGURE 2-3. Existing Information on Health Effects of 2,4,6-Trinitrotoluene



● Existing Studies

Recent literature pertaining to the health effects of 2,4,6-trinitrotoluene in humans described case reports and retrospective, intermediate- and chronic-duration studies of workers employed in the manufacture of ammunition. No acute human exposure data were located. For those employed in the manufacturing process using 2,4,6-trinitrotoluene, the major routes of exposure are dermal and inhalation. Thus, information on intermediate- and chronic-duration exposures comes exclusively from derrnal and inhalation exposure data. Information from occupational and retrospective studies is frequently limited by a lack of data regarding quantification of doses and precise duration of exposure. No information was located regarding developmental, reproductive, genotoxic, or cancer effects in humans by any route. Information on any effects in humans after oral exposure to 2,4,6-trinitrotoluene was also not found.

Virtually all of the data on animals regarding health effects from 2,4,6-trinitrotoluene exposure were obtained from studies in which 2,4,6-trinitrotoluene was administered orally. Over the past 25 years, the majority of the extensive studies done in several mammalian species (i.e., rat, mouse, and dog) were performed for the Army. One of those studies addresses the effects of 2,4,6-trinitrotoluene after derrnal exposure and intratracheal (performed under anesthesia) instillation.

Releases from hazardous waste sites or from military installations involved in the manufacture of 2,4,6-trinitrotoluene and the processing of munitions that contain the compound are the main sources of potential exposure of the general population to 2,4,6-trinitrotoluene. Because persons living in the vicinity of these two types of sites may be exposed by oral, dermal, or inhalation routes, additional information on the effects via these routes would be valuable.

2.9.2 Identification of Data Needs

Acute-Duration Exposure. Available data are not sufficient to derive acute oral or inhalation MRLs. There are no data regarding acute-duration exposure of humans to 2,4,6-trinitrotoluene by inhalation or oral routes. Additional studies in acute oral exposures to 2,4,6-trinitrotoluene are needed to determine the threshold level for neurological effects because the only available data demonstrate serious neurological effects in a single oral LD_{50} study in rodents (Dilley et al. 1982b). Populations

living in the vicinity of ammunition plants or hazardous waste disposal sites may be exposed to 2,4,6-trinitrotoluene. Exposure would most probably occur via the dermal route, but there is a possibility that inhalation and oral exposures may occur. A dermal patch test for 2,4,6-trinitrotoluene was positive in one case of contact dermatitis in a worker previously exposed to 2,4,6-trinitrotoluene (Goh 1988). In a week-long study of workers handling 2,4,6-trinitrotoluene, two metabolites (aminodinitrotoluenes) were present in the urine: 4-ADNT and 2-ADNT. No other effects of exposure were reported (Woolen et al. 1986). Renal effects were observed in mice and rats (discoloration of the urine) after oral exposure to a relatively high dose of 2,4,6-trinitrotoluene (Dilley et al. 1982b). Other signs of exposure were inactivity, tremors, and death in both species. Since populations living in the vicinity of ammunition plants may be exposed to 2,4,6-trinitrotoluene by all three routes, animal studies of exposure via all three routes would be useful in elucidating possible effects in humans after acute exposure.

Intermediate-Duration Exposure. One occupational intermediate-duration study of inhalation exposure to low levels of 2,4,6-trinitrotoluene in workers found a significant increase in the hepatic enzymes SGOT and LDH when the air concentration was increased from 0.3 to 0.8 mg/m^3 (Morton et al. 1976). Unspecified respiratory difficulties were reported in some workers. Hemoglobin levels remained within a normal range. Because of the very low exposure level, it is difficult to determine the major target organ since adaptation can mask changes in circulating hemoglobin levels. Another occupational report identified the immunological system as the target organ in a case of contact dermatitis (Goh and Rajan 1983). However, studies in laboratory animals following intermediateduration oral exposure did confirm the blood as a major target organ (Dilley et al. 1982b; Jiang et al. 1991; Levine et al. 1984, 1990a, 1990b). Other target organs identified in intermediate-duration oral studies include liver, kidney, gastrointestinal tract, spleen, central nervous system, and reproductive system (Dilley et al. 1982b; Levine et al. 1984, 1990a, 1990b). An intermediate-duration oral MRL for 2,4,6-trinitrotoluene has been calculated based on a study that noted dose-related liver effects in dogs receiving 0.5 mg/kg/day for 6 months (Levine et al. 1990b).

Chronic-Duration Exposure and Cancer. Few studies have been conducted on workers that have been chronically exposed to 2,4,6-trinitrotoluene. Increased levels of zinc and copper were found in hair samples of workers exposed for 3-7 years, mainly by the inhalation route (Jiang et al. 1991). Three other studies, two of inhalation (Harkonen et al. 1983; Savolainen et al. 1985) and one of dermal exposure (Harkonen et al. 1983), showed the occurrence of varying degrees of cataracts in exposed workers. Another effect after chronic inhalation exposure was the decreased activity of two mitochondrial enzymes necessary for heme synthesis: heme synthase and S-aminolevulinic acid synthase (Savolainen et al. 1985). More studies are needed to elucidate the meaning of that finding and its role in the possible development of anemia. One case of cirrhosis was reported after chronic occupational exposure (35 years) to 2,4,6-trinitrotoluene (Garfinkel et al. 1988). 2,4,6-Trinitrotoluene is known to cause hepatic damage, but it is not known whether it caused the cirrhosis,

The major adverse effects observed in Fisher rats fed 50 mg/kg/day for 24 months were anemia, hepatotoxicity and splenic lesions (Army 1984a). On the basis of this study's finding, chronicduration exposure to the intermediate-duration oral MRL of 0.0005 mg/kg/day (same value as the RfD) would not be anticipated to cause adverse health effects.

One case of hepatocellular carcinoma was reported after chronic occupational exposure (35 years) to 2,4,6-trinitrotoluene (Garfinkel et al. 1988). Carcinoma of the urinary bladder was observed in Fisher rats (in females only) fed 50 mg/kg/day for 24 months (Army 1984a). On the basis of these findings of bladder carcinoma, 2,4,6-trinitrotoluene was classified by EPA as a possible human carcinogen--Group C (EPA 1989b). Additional animal studies on chronic dermal and inhalation exposures would clarify if bladder carcinoma occurs after exposure by those routes, and if it is sex related.

Genotoxicity. Evidence that mutagenic substances are present in human urine comes from studies of occupationally exposed individuals (Ahlborg et al. 1985, 1988a). These exposures occurred via both inhalation and dermal routes, although the latter one appears to be more important in 2,4,6-trinitrotoluene uptake. The lack of a correlation between mutagenesis and 2,4,6-trinitrotoluene concentration in the urine of exposed workers is of considerable relevance and should be thoroughly investigated. The major metabolites of 2,4,6-trinitrotoluene are known to exert a mutagenic effect in

bacteria. It therefore appears likely that biotransformation products are responsible for the genotoxic activity in the urine of exposed workers since 2,4,6-trinitrotoluene concentrations are low. Similarly, the dose-response relationship between 2,4,6-trinitrotoluene exposure and the mutagenicity of exposed worker urine should be fully characterized in order to establish the urine assay as a reliable biomarker for exposure screens.

The limited whole animal studies provide assurance that 2,4,6-trinitrotoluene is not genotoxic in somatic cells. However, no information related to potential adverse effects on germinal cells was found; therefore, studies (e.g., dominant lethal mutation assay) should be considered to ensure that all relevant genetic end points have been investigated. There is reliable evidence that 2,4,6-trinitrotoluene induces mutations in bacterial (Army 1978a,c, 1979b, 1980c; Spanggord et al. 1982b) and mammalian cells (Styles and Cross 1983) and inconclusive evidence that 2,4,6-trinitrotoluene causes UDS in cultured human fibroblasts (Army 1978c). The relevance of these in vitro findings to human health should not be underestimated. The weight of evidence clearly suggests that the genotoxic activity of 2,4,6-trinitrotoluen(b is markedly inhibited or abolished in the presence of exogenous metabolic activation systems. Thus, the potential hazard to human genetic material resulting from exposure to 2,4,6-trinitrotoluene is very limited. It is, therefore, doubtful whether the performance of additional *in vitro* assays would substantially alter the established genetic toxicology profile of 2,4,6-trinitrotoluene. Confirmation of the mammalian cell assay findings is, nevertheless, desirable to establish full confidence in the validity of the existing data.

Reproductive Toxicity. Significantly lower semen volumes, a smaller percentage of motile spermatozoa, and a higher incidence of sperm malformation were reported in a case-control study in two 2,4,6-trinitrotoluene plants in China (Li et al. 1993). However, exposure to 2,4,6-trinitrotoluene was not estimated. No studies were found describing reproductive effects of 2,4,6-trinitrotoluene in human females. Studies in laboratory animals show dose-dependent reproductive toxicity after intermediate oral exposure to 2,4,6-trinitrotoluene. Testicular atrophy, degenerated germinal epithelium, and atrophic seminiferous tubules were effects observed in male rats after exposure to high doses of 2,4,6-trinitrotoluene (Levine et al. 1984). Additional studies of these effects after dermal and inhalation exposure would be helpful in determining if the effects are specific for the oral exposure

route. The results from one rat study indicate that there is a close correlation between reduction of testes weight and decreased zinc concentration (Jiang et al. 1991). The role of zinc and its possible effect on the male sex function needs further investigation. No studies were located that examined reproductive toxicity in females.

Developmental Toxicity. No human or animal studies were located on developmental effects for any exposure route. Studies in animals assessing postnatal survival after maternal exposure by all three routes would be useful.

Immunotoxicity. Very little information was located on immunological effects in humans after dermal and inhalation exposure to 2,4,6-trinitrotoluene. An increase in mononuclear leukocytes and lymphocytes was noted in a retrospective study of blood counts of exposed munitions workers (Army 1978a). The significance of this finding needs further investigation since no such data were presented in animal studies. Two isolated cases of allergic contact dermatitis were described in workers handling 2,4,6-trinitrotoluene (Goh 1988; Goh and Rajan 1983). The importance of 2,4,6-trinitrotoluene as an allergen needs to be examined further in order to understand the mechanisms involved in the possible development of hypersensitivity to 2,4,6-trinitrotoluene.

Dose-dependent immunological reactions were seen in mice, rats, and dogs after intermediate oral exposure to 2,4,6-trinitrotoluene. Spleen enlargement and/or increased weight was the most often observed effect (Dilley et al. 1982b; Levine et al. 1984, 1990a). Histopathology revealed hemosiderosis and varying degrees of splenic congestion. Further studies of immunological effects in animals after chronic exposure would be important for estimating human susceptibility for populations potentially exposed in the vicinity of ammunition plants.

Neurotoxicity. Limited information regarding neurological effects in humans indicates only minor effects such as altered taste (Morton et al. 1976). Dose-related changes in behavior were observed in several animal species after acute and intermediate oral exposure to 2,4,6-trinitrotoluene. Most common were depression (Dilley et al. 1982b), lethargy, and ataxia (Levine et al. 1990b). Brain lesions were present in rats receiving the highest dose of 2,4,6-trinitrotoluene (Dilley et al. 1982b;

Levine et al. 1984). The results also show species differences in 2,4,6-trinitrotoluene-induced neurotoxicity, with dogs being more sensitive than rats or mice. Dose-response studies in animals and studies focusing on the mechanism of 2,4,6-trinitrotoluene-induced neurotoxicity would be useful in better understanding the possible neurotoxicity of 2,4,6-trinitrotoluene in humans.

Epidemiological and Human Dosimetry Studies. Human studies on 2,4,6-trinitrotoluene consist of either retrospective studies of occupational exposure or case reports of workers employed in the manufacture of munitions. Exposures in both cases are primarily dermal and by inhalation. Locating populations for future epidemiological studies will be possible as long as 2,4,6-triaitrotoluene is produced and used in the manufacture of munitions. The two subgroups with the greatest possible exposure to 2,4,6-trinitrotoluene are those employed in the manufacturing process and those living in the vicinity of military installations/ammunition plants. If such groups are located, information regarding the immunologic, reproductive, developmental, genotoxic, and cancer effects and correlation of these effects with blood or urine levels of 2,4,6-trinitrotoluene that are associated with exposure would be extremely useful.

Biomarkers of Exposure and Effect. Exposure to 2,4,6-trinitrotoluene is currently measured by determining the level of 2,4,6-trinitrotoluene in the blood or urine. However, since 2,4,6-trinitrotoluene is rapidly metabolized it may be difficult to determine trace amounts of unchanged compound in either blood or urine. In such cases, the presence of major 2,4,6-trinitrotoluene metabolites such as 4-ADNT and 2-ADNT metabolites, which are present in the urine for over 2 weeks after acute exposure, can be used to indicate recent and past exposures (Woolen et al. 1986). Although the sensitivity of these biomarkers seems to be sufficient at the present time, it would be useful to determine the metabolite responsible for urine color change that occurs after exposure to 2,4,6-trinitrotoluene. Identification of this metabolite in urine would provide an early biomarker of 2,4,6-trinitrotoluene exposure. Since 2,4,6-trinitrotoluene has been found to bind to blood and liver proteins, the identity of these adducts and their tissue lifetimes would greatly enhance the use of these biomarkers as a measure of exposure.

Decreases in hemoglobin and hematocrit levels and increases in reticulocyte numbers can be monitored and accurately determined after exposure to 2,4,6-trinitrotoluene. In some cases of prolonged exposure, these changes lead to the development of an anemic state. However, the changes in these blood parameters are not specific for exposure to 2,4,6-trinitrotoluene. What would be useful is a better understanding of the fundamental mechanism by which 2,4,6-trinitrotoluene causes changes in hemoglobin and hematocrit levels. Such an understanding might ultimately lead to the development of antidotes to decrease or completely alleviate some of the toxic effects caused by 2,4,6-trinitrotoluene.

There are no tests that are specific for 2,4,6-trinitrotoluene-induced hepatic toxicity, but standard liver function tests should be able to identify hepatic toxicity caused by 2,4,6-trinitrotoluene. Although not specific for 2,4,6-trinitrotoluene exposure, more information is needed on changes in indicators of 2,4,6-trinitrotoluene-induced hepatotoxicity, for example, SGOT and LDH levels.

Jaundice may develop as a result of 2,4,6-trinitrotoluene exposure. Since jaundice is a late phenomenon, it is important to be sure that it is due to serious hepatic injury and not merely to the yellowing of the body's surface exposed to the compound.

Cataract formation is believed to be a specific 2,4,6-trinitrotoluene effect, developing primarily after chronic exposures (Harkonen et al. 1983; Savolainen et al. 1985). Since initial changes are small and often difficult to detect, development of more sensitive detection techniques would allow for earlier detection and prevention of potential adverse ocular effects due to 2,4,6-trinitrotoluene exposure. This is important because in the course of chronic occupational exposure, cataract formation may be the only sign of 2,4,6-trinitrotoluene toxicity. Additional studies are also needed to establish if cataract formation is a dose-response phenomenon.

Absorption, Distribution, Metabolism, and Excretion. The majority of information regarding the rapid absorption of 2,4,6-trinitrotoluene in humans and animals comes indirectly from detectable levels of 2,4,6-trinitrotoluene metabolites in the urine after inhalation, oral, or dermal exposures. Occupational studies indicate that humans readily absorb 2,4,6-trinitrotoluene dusts via inhalation or dermal contact, but quantitative data are lacking. Animal studies indicate that 2,4,6-trinitrotoluene is

absorbed relatively quickly and that the absorbed amount is dose related for the oral route (Army 1981d). Information concerning absorption rates for all three routes is needed. Because there are few absorption studies for all three routes, additional quantitative data in animals would be useful as a basis for estimates of absorption in humans.

No studies were located regarding distribution following inhalation, oral, or dermal exposure to 2,4,6-trinitrotoluene in humans; limited information is available regarding distribution in animals after acute exposure to radiolabelled 2,4,6-trinitrotoluene via all three routes. These studies in animals indicate that 2,4,6-trinitrotoluene is rapidly distributed to blood, liver, fat, and skeletal muscle, but in very small amounts because the majority of the label was recovered from the gastrointestinal tract and urine (Army 1981d). Additional animal distribution studies on dermal exposure would be valuable to establish the biological half-lives in relevant tissues and because there is a potential for human exposure to occur via this route.

No studies were located regarding the metabolism of 2,4,6-trinitrotoluene in humans after oral exposure. Data for the oral route are important because there is a potential for human exposure to occur via this route near waste sites containing 2,4,6-trinitrotoluene. Limited information comes from the analysis of urine of munitions workers after dermal (Woolen et al. 1986) or inhalation exposures (Hassman and Hassmanova 1976). The results from human and animal studies indicate that 2,4,6-trinitrotoluene is readily metabolized and that very small amounts of unchanged compound are present in the urine. More studies are needed to define sex-related and species-related metabolic differences. Since differences in metabolism may occur with differences in the route of exposure, additional information is needed from inhalation and dermal metabolic studies in order to fully characterize the metabolic pathway of 2,4,6-trinitrotoluene.

No studies were located regarding excretion after oral or dermal exposure to 2,4,6-trinitrotoluene in humans. The results from animal studies indicate that urine is the major site of radiolabel recovery after a single oral dose of 2,4,6-trinitrotoluene and that differences in excretion rate are exposure routedependent (Army 1981d). The recovery of radiolabel indicates that excretion was most efficient after inhalation exposure, followed by oral, and was least efficient after dermal exposure. Additional, more

detailed studies on excretion after dermal exposure would be useful since that is an expected major exposure route for humans in the vicinity of waste sites and workers handling 2,4,6-trinitrotoluene.

Comparative Toxicokinetics. Studies using different animal species (rats, mice, dogs, rabbits) indicate the kinetics of 2,4,6-trinitrotoluene differ across species. The observed differences are primarily quantitative (Army 1981b). On the basis of kinetic data alone, it is not possible to identify target organs common to humans and animals, but distribution data together with toxicity data after oral exposure suggest similar target organs: blood, liver, spleen, the kidneys and gastrointestinal tract. Interspecies differences, especially in metabolism and excretion have been noted in rats, mice, dogs, and rabbits (Army 1981b). Further animals studies covering all three exposure routes would be helpful in determining similarities and differences in absorption, metabolism and excretion between humans and animals.

Methods for Reducing Toxic Effects. Animal studies addressing the possibility of diminishing or alleviating toxic effects of 2,4,6-trinitrotoluene are needed since no information was located on possible 2,4,6-trinitrotoluene antidotes.

2.9.3 On-going Studies

On-going studies regarding the health effects of 2,4,6-trinitrotoluene were reported in the Federal Research in Progress File (FEDRIP 1991) database. One study addressing health effects of 2,4,6-trinitrotoluene in humans, “Deposition of volatile aerosols in the respiratory tract,” is being investigated at the University of Rochester, Rochester, New York, by Sidney Soderholm, principal investigator. The sponsoring organization is the National Institute of Environmental Health Sciences,

No additional studies have been reported in the FEDRIP 1994, but it is likely that research in China on industrial exposure will continue (Li et al. 1991, 1992, 1993). The studies in Germany of populations surrounding former munitions manufacturing and disposal areas are being refined (Kolb et al. 1993).

3. CHEMICAL AND PHYSICAL INFORMATION

3.1 CHEMICAL IDENTITY

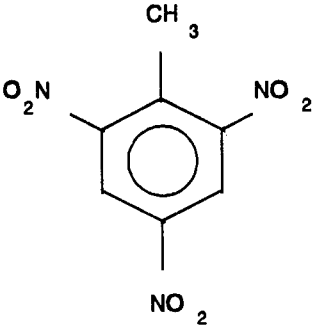
Information regarding the chemical identity of 2,4,6-trinitrotoluene is located in Table 3- 1.

3.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of 2,4,6-trinitrotoluene is located in Table 3-2.

CHEMICAL AND PHYSICAL INFORMATION

TABLE 3-1. Chemical Identity of 2,4,6-Trinitrotoluene

Characteristic	Information	Reference
Chemical name	2,4,6-Trinitrotoluene	HSDB 1990
Synonym(s)	sym-trinitotoluene; 1-methyl-2,4,6-trinitrobenzene; 2-methyl-1,3,5-trinitrobenzene; alpha-TNT; TNT; alpha-trinitrotoluol; tolit; tritol; trotyl oil; trilit	HSDB 1990
Registered trade name(s)	No data	
Chemical formula	$C_7H_5N_3O_6$	Budavari et al. 1989
Chemical structure		Sax and Lewis 1987
Identification numbers:		
CAS registry	118-96-7	Budavari et al. 1989
NIOSH RTECS	XU0175000	HSDB 1990
EPA hazardous waste	No data	
OHM/TADS	7217371	HSDB 1990
DOT/UN/NA/IMCO shipping	TNT, dry or wetted with <30% water (UN 0209/IMO 1.1) TNT, wetted with >30% water (UN 1356/IMO 4.1)	HSDB 1990
HSDB	1146	HSDB 1990
NCI	C56155	HSDB 1990

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

TABLE 3-2. Physical and Chemical Properties of 2,4,6-Trinitrotoluene

Property	Information	Reference
Molecular weight	227.13	Budavari et al. 1989
Color	Yellow	Budavari et al. 1989
Physical state	Monoclinic needles	Budavari et al. 1989
Melting point	80.1°C	Budavari et al. 1989
Boiling point	240°C (explodes)	HSDB 1990
Specific gravity	1.654	Budavari et al. 1989
Odor	Odorless	NIOSH 1990
Odor Threshold:		
Water	No data	
Air	No data	
Solubility:		
Water at 20°C	130 mg/L	HSDB 1990
Organic solvent(s)	Soluble in acetone and benzene; soluble in alcohol and ether	Budavari et al. 1989
Partition coefficients:		
Log K_{ow}	1.60; 2.2 (measured)–2.7 (estimated)	HSDB 1990; Spanggord et al. 1985
K_{oc}	300 (estimated)–1,100 (measured)	Spanggord et al. 1985
Vapor pressure at 20°C	1.99×10^{-4} mmHg	HSDB 1990
Henry's law constant:		
at 20°C	4.57×10^{-7} atm m^3/mole	HSDB 1990
at 30°C	No data	HSDB 1994
Autoignition temperature	No data	HSDB 1994
Flashpoint	Explodes	NIOSH 1994
Flammability and Reactivity	4.4	HSDB 1994
Conversion factors	1 ppm = 9.28 mg/m^3 1 mg/m^3 = 0.108 ppm	NIOSH 1973
Explosive temperature	464°F	HSDB 1994
Explosive limits	No data	NIOSH 1990

4. PRODUCTION, IMPORT, USE, AND DISPOSAL

4.1 PRODUCTION

2,4,6-Trinitrotoluene is prepared by the nitration of toluene with a mixture of nitric acid and sulfuric acid (Fisher and Taylor 1983; Sax and Lewis 1987). Toluene is nitrated in a three-step operation by using increasing temperatures and mixed-acid concentrations to successively introduce nitro groups to form mononitrotoluene (MNT), dinitrotoluene (DNT), and trinitrotoluene (Mark et al. 1980). The nitration can be accomplished in three separate steps or by continuous flow (Budavari et al. 1989). Numerous other compounds are also formed during the nitration of toluene including unsymmetrical isomers of 2,4,6-trinitrotoluene, and oxidation products such as tetranitromethane, nitrobenzoic acid, nitrocresol, and partially nitrated toluenes (Hamilton and Hardy 1974; Mark et al. 1980). The unsymmetrical 2,4,6-trinitrotoluene isomers are removed by washing with aqueous sodium sulfite solution (Fisher and Taylor 1983; Mark et al. 1980; Sax and Lewis 1987).

2,4,6-Trinitrotoluene is not produced commercially in the United States; production is limited to military arsenals (HSDB 1994). Data on production volumes for 2,4,6-trinitrotoluene are not available. 2,4,6-Trinitrotoluene is purchased from the U.S. Army Armament Material Command (Gibbs and Popolato 1980). Army ammunition plants that have been involved in the production and storage of 2,4,6-trinitrotoluene include Shreveport (Louisiana), Anniston (Alabama), Crane (Indiana), Fort Wingate (New Mexico), Hawthorne (Nevada), Letterkenny (Pennsylvania), Lexington (Kentucky), McAlester (Oklahoma), Navajo (Arizona), Pine Bluff (Arkansas), Pueblo (Colorado), Red River and Lone Star (Texas), Savanna and Joliet (Illinois), Seneca (New York), Sierra (California), Tooele (Utah), and Umatilla (Oregon) Weldon Spring (Missouri), West Virginia Ordnance Works (West Virginia), Radford (Virginia), and Volunteer (Tennessee) (Army 1986a, 1986d; Haroun et al. 1990; Kraus et al. 1985; Phung and Bulff 1981).

Since 2,4,6-trinitrotoluene releases are not required to be reported under SARA Section 313, there are no data on 2,4,6-trinitrotoluene in the 1988 Toxics Release Inventory (TRI88 1990).

4.2 IMPORT/EXPORT

In 1985, an estimated 9.2 million pounds of 2,4,6-trinitrotoluene were imported into the United States (USDOC 1986). However, current import and export data for 2,4,6-trinitrotoluene are not available.

4.3 USE

2,4,6-Trinitrotoluene has been classified as a high explosive (Eveleth and Kollonitsch 1990). The compound is used as a military explosive in bombs and grenades (HSDB 1994; OHM/TADS 1985). It has been widely used for filling shells and airborne demolition bombs since it is sufficiently insensitive to the shock of ejection from a gun barrel but can be exploded on impact by a detonator mechanism (Eveleth and Kollonitsch 1990). 2,4,6-Trinitrotoluene has been used either as the pure explosive or in binary mixtures (Gibbs and Popolato 1980). The most common binary mixtures of 2,4,6-trinitrotoluene are cyclotols (mixtures with RDX), octols (mixtures with HMX), amatols (mixtures with ammonium nitrate), and tritonals (mixtures with aluminum) (Eveleth and Kollonitsch 1990; Gibbs and Popolato 1980). In addition to military use, small amounts of 2,4,6-trinitrotoluene may be used for industrial explosive applications, such as deep well and underwater blasting (HSDB 1994).

Other industrial uses of 2,4,6-trinitrotoluene include use as a chemical intermediate in the manufacture of dyestuffs and photographic chemicals (Sax and Lewis 1987).

4.4 DISPOSAL

Wastes generated in the manufacture of 2,4,6-trinitrotoluene are characterized as hazardous wastes by EPA, and EPA regulations for disposal must be followed (EPA 1990). For more information on the regulations that apply to 2,4,6-trinitrotoluene, see Chapter 7.

Disposal of 2,4,6-trinitrotoluene has been accomplished effectively by burning in an incinerator equipped with an afterburner and a scrubber (OHM/TADS 1985). 2,4,6-Trinitrotoluene has been pretreated before incineration by pouring or sifting onto sodium bicarbonate or a sand-soda ash

mixture. The resulting combination is mixed and packaged in heavy paper cartons with plenty of paper packaging to serve as fuel (OHM/TADS 1985). 2,4,6-Trinitrotoluene has also been prepared for incineration by mixing with a flammable solvent, such as alcohol or benzene, and spraying the resulting mixture into the fire chamber of an incinerator (OHM/TADS 1985).

Demilitarization is the use of various technologies to process munitions so they are no longer suitable for military applications. Demilitarization of munitions involves a number of techniques. Both destructive and nondestructive methods are used. Destructive methods include incineration, open detonation, and open burning. Nondestructive methods are aimed at recovering various components for reuse or sale. Destructive methods are the most predominant type used at the various depots and ammunition plants across the country (Army 1986). Munitions are demilitarized because of obsolescence of weapons, deterioration of chemical components, and poor serviceability. 2,4,6-Trinitrotoluene is the primary explosive filler in the demilitarization inventory.

Bench-scale cornposting of up to 10% 2,4,6-trinitrotoluene showed that almost complete removal of 2,4,6-trinitrotoluene occurred in 55 days, and many of the toxic transformation products formed in activated sludge and soil were not found in the composted 2,4,6-trinitrotoluene (Fisher and Taylor 1983). Field studies have demonstrated composting is effective in removing 2,4,6-trinitrotoluene from contaminated lagoon sediments under both thermophilic and mesophilic conditions (Williams et al. 1992). The mutagenicity of the metabolites of 2,4,6-trinitrotoluene formed by cornposting was found to be less than the parent compound (Tan et al. 1992). Waste water contaminated with 2,4,6-trinitrotoluene and various concentrations of nitrobodies, such as RDX, was successfully oxidized electrochemically from a range of 60-105 ppm 2,4,6-trinitrotoluene to below acceptable disposal concentrations (0.5 ppm); the oxidation process did not produce any toxic by-products (HSDB 1994). Other methods of treating waste waters contaminated with 2,4,6-trinitrotoluene and related products that have been investigated include ultrafiltration, activated carbon, and resin adsorption (EPA 1982).

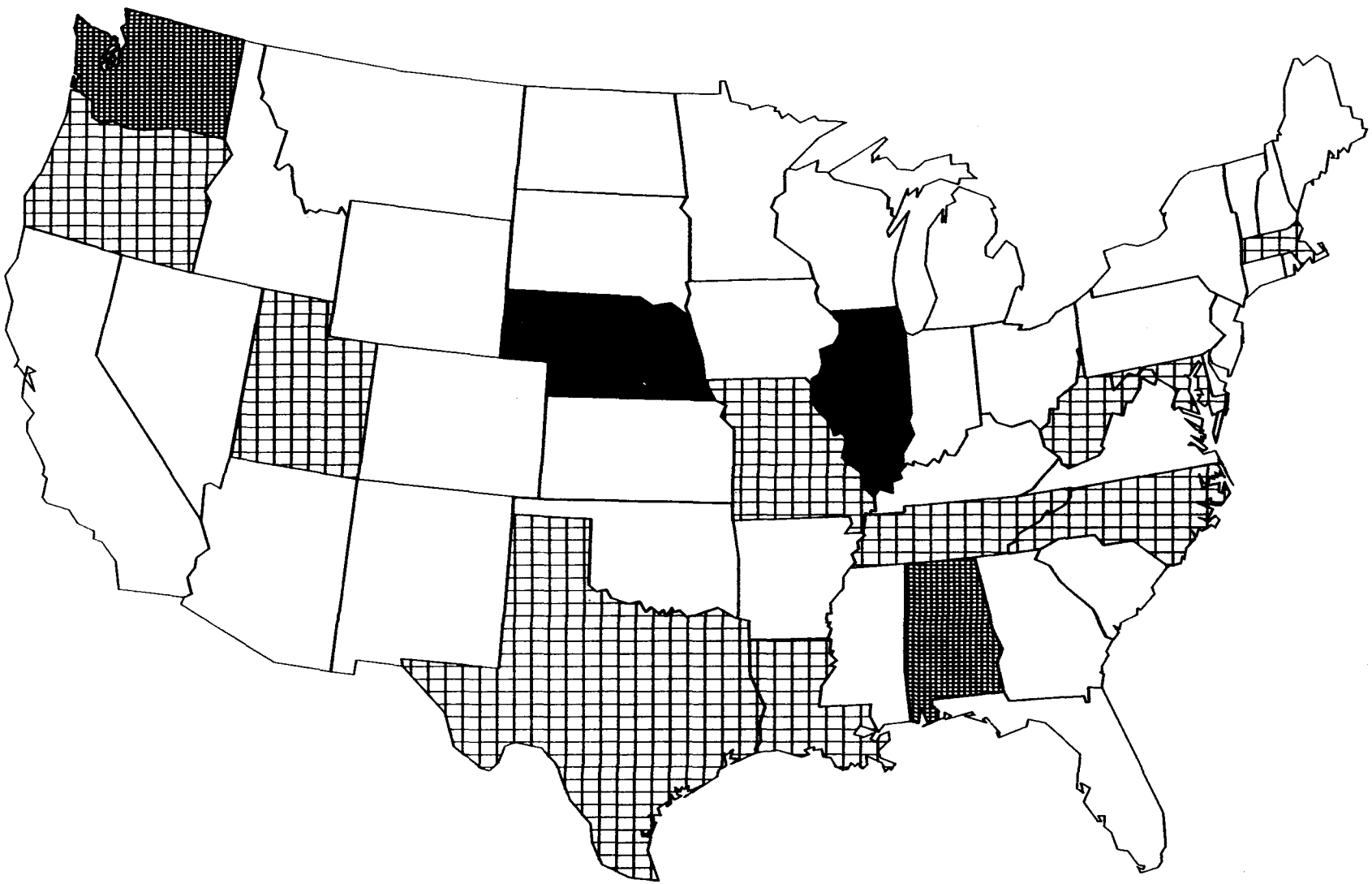
5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

2,4,6-Trinitrotoluene is released to the environment from manufacturing and munitions processing facilities. Upon release to surface waters, 2,4,6-trinitrotoluene undergoes rapid photolysis to a number of products. Biodegradation by microorganisms including bacteria and fungi also occurs in surface waters but at rates much slower than photolysis. 2,4,6-Trinitrotoluene is expected to be transported mainly in the aqueous phase; the compound is not expected to volatilize from surface water to the atmosphere or significantly partition to soils or sediments. Bioconcentration of 2,4,6-trinitrotoluene by plants and aquatic organisms is limited, and biomagnification of the compound in terrestrial and aquatic food chains is not expected. Little information is available on the concentrations of 2,4,6-trinitrotoluene or its degradation products in ambient media. The most important routes of human exposure to the compound, that the general public may be exposed to, appear to be ingestion of contaminated drinking water and dermal contact with contaminated surface water. However, members of the general public may also be exposed to 2,4,6-trinitrotoluene released to the atmosphere as a result of ordnance demilitarization and disposal through incineration and detonation, as well as by ingestion of foods contaminated through uptake of the compound from contaminated soils or deposition of 2,4,6-trinitrotoluene particulates from the atmosphere. Workers may be exposed via inhalation and dermal contact. Workers involved in the manufacture of 2,4,6-trinitrotoluene or the processing of munitions containing the compound may be exposed to high concentrations of 2,4,6-trinitrotoluene through inhalation and dermal contact.

2,4,6-Trinitrotoluene has been identified in at least 20 of the 1,397 hazardous waste sites on the EPA National Priorities List (NPL) (HAZDAT 1994). However, the number of sites evaluated for 2,4,6-trinitrotoluene is not known. The frequency of these sites within the United States can be seen in Figure 5-l.

FIGURE 5-1. FREQUENCY OF NPL SITES WITH 2,4,6-TRINITROTOLUENE CONTAMINATION *



*Derived from HazDat 1994

5.2 RELEASES TO THE ENVIRONMENT

5.2.1 Air

2,4,6-Trinitrotoluene is released to the ambient atmosphere as a result of open detonation and open burning techniques used in the demilitarization of munitions (Army 1986e) (also see Section 4.4). Gases and particulates are released to the atmosphere as a result of these activities and from the disposal of munitions containing 2,4,6-trinitrotoluene in rotary kiln incinerators. 2,4,6-Trinitrotoluene dusts and vapor are released into indoor air atmospheres in military production and processing facilities during manufacturing of 2,4,6-trinitrotoluene and munitions (Hathaway 1985). Fugitive dusts containing the compound are probably generated at sites with contaminated surface soils (e.g., military installation burning grounds) (Kraus et al. 1985).

5.2.2 Water

2,4,6-Trinitrotoluene has historically been discharged in large quantities in the aqueous effluents of explosives production/manufacturing facilities and ammunition load, assemble, and pack (LAP) plants, from decommissioning activities, and through field use/disposal. Estimates of the loadings of 2,4,6-trinitrotoluene in these effluents vary. Some investigators have reported concentrations of about 120 mg/L in manufacturing facility waste waters and 25 mg/L in loading plant facility effluents (Freeman and Colitti 1982). Others report concentrations of 40-120 mg/L in manufacturing plant effluents, with generally higher concentrations in LAP plant waste waters (Andren et al. 1977b). Concentrations of 0.1-3.4 mg/L have been detected in about 20% of the samples of sellite manufacturing process condensate wastewater collected from a 2,4,6-trinitrotoluene manufacturing facility (Army 1980b; Spanggord et al. 1982a).

Estimates of historical 2,4,6-trinitrotoluene releases to surface waters from Army ammunition plants have been developed on the basis of surveys of munitions facilities. Sources include production plants (emissions range from 61 to 210 pounds/day) and LAP plants (l-150 pounds/day). Estimates of average downstream concentrations of 2,4,6-trinitrotoluene in the surface waters receiving these

effluents are 0.006-0.025 mg/L for production plants and <0.001-0.038 mg/L for LAP plants (Rosenblatt et al. 1973; Small and Rosenblatt 1974). 2,4,6-Trinitrotoluene has been detected in the effluent of the Radford, Virginia, production plant at 101-143 ppm (Nay 1972a).

5.2.3 Soil

2,4,6-Trinitrotoluene is released to soils from spills, disposal of solid waste, open incineration and detonation of ordnances, leaching from inadequately sealed impoundments (e.g., pits, ponds, and lagoons), and demilitarization of munitions (EPA 1989c; Kraus et al. 1985; Army 1986e). Demilitarization of munitions can result in contamination of surface soils by activities such as open burning and open detonation or landfilling of solid wastes generated during rotary kiln incineration and nondestructive reprocessing of munitions containing 2,4,6-trinitrotoluene (Army 1986e).

5.3 ENVIRONMENTAL FATE

5.3.1 Transport and Partitioning

On the basis of the relatively low vapor pressure ($1.99x10^{-4}$ mmHg at 20°C) and relatively high water solubility (130 mg/L at 20°C) (see Table 3-2) of 2,4,6-trinitrotoluene, the compound is not expected to partition from surface waters to the atmosphere. Limited volatilization from aqueous solutions was found in air stripping tests on raw and neutralized waste water samples, where only 8-10% of the 2,4,6-trinitrotoluene concentration was lost during an 18-day test period (Nay 1972a). Volatilization half-lives of 10,000 days have been estimated for ponds, streams, and lakes (Spanggord et al. 1985). A volatilization half-life of 119 days has been estimated from a model river at 20°C 1 meter deep flowing at the rate of 1 meter/second, with a wind speed of 3 meters/second (HSDB 1994).

On the basis of the measured and estimated values for the soil organic carbon adsorption coefficient (K_{oc}) of 300-1,100, 2,4,6-trinitrotoluene is not expected to significantly partition from surface waters to sediment or strongly sorb to soil particulates (Spanggord et al. 1985). This expected behavior has been confirmed in short-term laboratory adsorption/desorption tests and long-term lysimeter studies. Short-term (24-hour) laboratory batch adsorption/desorption tests were conducted using

uncontaminated surface soils collected from 13 Army ammunition plants. Limited 2,4,6-trinitrotoluene adsorption was found in these tests. The average adsorption coefficient (K_d) for all soils tested was 4, which indicates limited sorption potential. Adsorption was found to be consistently lower under oxidized conditions than under reduced conditions. Almost all of the 2,4,6-trinitrotoluene adsorbed was desorbed upon multiple extraction of the test soils. The pH of the soils exerted no effect on 2,4,6-trinitrotoluene adsorption/desorption or transformation. Transformation products (4-ADNT and 2-ADNT) were detected under both oxidized and reduced conditions (Pennington and Patrick 1990). In long-term lysimeter studies, ring-labeled ^{14}C-2,4,6-trinitrotoluene was added to the top 3 inches of soils in eolmnns (2 feet long and 2 inches in diameter). Four soil types were used, ranging in texture from fine to coarse. The lysimeters were regularly irrigated during the 6-month test period, and column leachate samples were taken every 2 weeks. At the end of the test period, the soil columns were sectioned for analysis. 2,4,6-Trinitrotoluene and its transformation products were retained in the test soil columns. Neither 2,4,6-trinitrotoluene nor its typical biodegradation products were detected in the leachate samples. Analysis of the leachate samples with high ^{14}C activity indicated the presence of only highly polar, nonvolatile products. These products could not be separated or identified. However, two transformation products were identified in the soil columns: 2-ADNT and 4-ADNT. The concentration of these products in the soil columns ranged from 0.01% to 6% of the radiolabelled 2,4,6-trinitrotoluene added to the columns (Kayser and Burlinson 1988).

In other mobility tests with sediments, ring-labeled ^{14}C-2,4,6-trinitrotoluene was added to unsterilized sediments collected from two farm ponds in Syracuse, New York and from the Holston River in Kingsport Tennessee, upstream from an Army Ammunition Plant site. 2,4,6-Trinitrotoluene was not extensively sorbed in short-term (24 hour) tests, partition coefficients varied with pH and temperature. Desorption of 2,4,6-trinitrotoluene or its breakdown products proceeded slowly; steady state conditions were reached after 92 hours in only 1 sediment (Army 1980b).

The log octanol/water partition coefficient (K_{ow}) values of 2.2-2.7 (see Table 3-2) suggest that the compound will not bioconcentrate to high levels (i.e., concentrations ≥ 1,000 times media concentrations) in the tissues of exposed plants and animals or biomagnify in terrestrial or aquatic food chains (Spanggord et al. 1985). Limited bioconcentration was demonstrated in aquatic bioassays with

water fleas *(Daphnia magna),* worms *(Lumbriculus variegatus),* algae *(Selenastrum capricornutum),* and bluegill sunfish *(Lepomis macrochirus).* Bioconcentration factors (BCFs) in 96-hour static tests were found to be 209 for the water flea, 202 for the worms, 453 for algae, 9.5 for fish muscle, and 338 for fish viscera (Liu et al. 1983b).

Bioconcentration of 2,4,6-trinitrotoluene by yellow nutsedge was studied in hydroponic cultures containing 5, 10, and 20 mg/L 2,4,6-trinitrotoluene. After a 42-day exposure period, the rhizomes, roots, tubers, and leaves of the plants were analyzed for 2,4,6-trinitrotoluene and metabolites. 2,4,6-Trinitrotoluene and its metabolites, 4-ADNT and 2-ADNT, were taken up and translocated throughout the plants, although the highest concentrations were found in the roots. Concentrations of 2,4,6-trinitrotoluene and metabolites in plant tissues generally increased with increasing 2,4,6-trinitrotoluene concentrations in the growth medium. At the 20-mg/L treatment level, the concentrations in plant roots were 714 mg/kg, 614 mg/kg, and 2,180 mg/kg for 2,4,6-trinitrotoluene, 2-ADNT, and 4-ADNT, respectively (Palazzo and Leggett 1986).

5.3.2 Transformation and Degradation

5.3.2.1 Air

No information was found on the transformation of 2,4,6-trinitrotoluene in the atmosphere. However, 2,4,6-trinitrotoluene released to the atmosphere should undergo direct photolysis, as it does in surface water. Estimates of the photolytic half-life of the compound in air range from 3.7 to 11.3 hours; these estimates are based on the rate of photolysis of the compound in distilled water. Estimates of the photooxidation half-life of the compound in the atmosphere range from 18.4 to 184 days. These estimates are based on the estimated rate constant for reaction with hydroxyl radicals in the atmosphere (Howard et al. 1991).

5.3.2.2 Water

2,4,6-Trinitrotoluene does not undergo hydrolysis, as demonstrated by the stability of the compound in sea water after 108 days at room temperature (Hoffsommer and Rosen 1973).

Photolysis of 2,4,6-trinitrotoluene in aqueous solutions is a well-known phenomenon, which is responsible for the development of "pink water," and is probably the most important fate process for 2,4,6-trinitrotoluene in aqueous systems. For example, the estimated half-life of 2,4,6-trinitrotoluene in surface waters is 0.16-1.28 hours, based on the rate of photolysis and photooxidation in sunlit natural waters (Howard et al. 1991). The rate of photolysis of 2,4,6-trinitrotoluene in natural surface waters has been found to be much greater than that of the compound in pure water. Phototransformation of 2,4,6-trinitrotoluene in surface waters occurs via direct and indirect photolysis. Direct photolysis of the compound is rapid; the estimated half-life varies from 14 to 84 hours, depending on season and latitude. These rates are increased in natural waters through the influence of humic acids on indirect photolysis. In sunlit natural waters, 2,4,6-trinitrotoluene photolysis proceeds at rates 10-100 times more rapid than those found in distilled water, with half-lives in some natural waters of less than 0.5 hour. Phototransformation in natural surface waters may be accelerated because of the complexation of 2,4,6-trinitrotoluene and natural organics, or by an indirect mechanism by which light absorbed by natural organic constituents is transferred to 2,4,6-trinitrotoluene, or by the chemical trapping by humic acids of the reactive intermediate phototransformation products (Mabey et al. 1983; Spanggord et al. 1985; Zepp et al. 1984). In laboratory studies using distilled water, the rate of transformation increases over time, since photolysis is also promoted by the presence of photodecomposition products in the medium. The pH of the surface water has been found to exhibit a small influence on the rate of transformation only in surface waters that contain few natural organic constituents. 2,4,6-Trinitrotoluene may be more persistent in deep quiescent water bodies or other water systems where sunlight is attenuated. A number of 2,4,6-trinitrotoluene photodecomposition products have been identified, including dinitroanthrils, trinitrobenzaldehyde, trinitrobenzyl alcohol, trinitrobenzene, nitroanilines, condensed azo and azoxy derivatives, and 1,3,5-trinitrobenzene (Burlinson 1980; Mabey et al. 1983). Recently a deep red-brown 2,4,6-trinitrotoluene degradation product of *Mycobacterium* grown in aerobic conditions has been identified (Vorbeck et al. 1994). This

type of compound (a hydride-Meisenheimer complex) may be the type of chemical causing "pink water."

2,4,6-Trinitrotoluene is also transformed in surface waters by microbial metabolism, although this process occurs more slowly than photolysis. For example, the estimated biodegradation half-life of 2,4,6-trinitrotoluene in surface water, under both aerobic and anaerobic conditions, is l-6 months. This estimate is based on aerobic river die-away test data with unacclimated microorganisms (Howard et al. 1991). The relative slowness of microbial degradation may be due in part to the enhanced toxicity of 2,4,6-trinitrotoluene to aquatic organisms in the presence of the near-ultraviolet component of sunlight (Johnson et al. 1994a). Examples of biotransformation of the compound in aqueous systems include the white rot fungus *Phanerochaete chrysosporium,* which was found to degrade ringlabeled ^{14}C-2,4,6-trinitrotoluene. Within 12 days, 35% of the labeled 2,4,6-trinitrotoluene added to the solution was recovered as $^{14}CO_2$ (Fernando et al. 1990).

Pseudomonad bacteria *(Pseudomonas* sp.) have been found to reduce 2,4,6-trinitrotoluene under aerobic conditions in laboratory studies to monoaminodinitrotoluenes and a diaminomononitrotoluene (Schackmann and Muller 1991). Pseudomonads isolated from mud and water samples collected at the U.S. Naval Ammunition Depot at McAlester, Oklahoma, have also been found to be capable of biotransforming 2,4,6-trinitrotoluene in laboratory studies. 2,4,6-Trinitrotoluene degraded most rapidly in cultures supplemented with yeast extract. In the most active isolate, complete dissimilation was found within 24 hours. Degradation products identified include 2,2′6,6′-tetranitro-4,4′-azoxytoluene; 9,4′,6,6′-tetranitro-2,2′-azoxytoluene; 2-amino-4,6-dinitrotoluene; 4-hydroxylamino-2,6-dinitrotoluene; and nitrodiaminotoluene (Won et al. 1974). Pseudomonads isolated from Narragansett Bay, sediments, raw sewage, and boiler plant effluents were able to utilize ring labeled ^{14}C-2,4,6-trinitrotoluene as a sole carbon source in laboratory degradation studies. The amount of 2,4,6-trinitrotoluene transformation varied with the concentration of the test compound in the medium. 2,2′6,6′-Tetranitro-4,4′-azoxytoluene was the only transformation product identified. Nitrite was found in the test medium, which suggests that the transformation proceeded via removal of the nitro groups from the aromatic ring. The recovery of 0.8-1.2% of the label in the form of $^{14}CO_2$ suggests a mechanism that includes cleavage of the aromatic ring (Traxler et al. 1974).

Microbial inocula isolated from sewage treatment plant effluents, waste water from a 2,4,6-trinitrotoluene ordnance loading facility, soil suspension, pond water, and aquarium water were found to be capable of degrading 2,4,6-trinitrotoluene in the presence of yeast extract in shake flask cultures at 38°C over 6 days. 2,4,6-Trinitrotoluene concentrations were reduced from the initial loading of 100 mg/L to 0-6 mg/L over the 6-day incubation period. Transformation did not occur in cultures containing only 2,4,6-trinitrotoluene and mineral salts. Microbial inocula isolated from raw sewage were not effective in transforming 2,4,6-trinitrotoluene; however, inocula isolated from sewage sludge digester liquor (supematant) reduced 2,4,6-trinitrotoluene concentrations by 64% over the test period. 2,4,6-Trinitrotoluene was also degraded in tests with a pure culture of *Pseudomonas aeruginosa* when glucose and supplemental nitrogen in the form of mineral salts were added to the culture medium (Osmon and Klausmeier 1973).

Sediments from Army ammunition plants containing mixtures of explosives, including 2,4,6-trinitrotoluene, have been composted in field trials to reduce their explosives content. For example, sediment from a Louisiana Army Ammunition Plant containing mixed explosives, including 56,800 mg/kg 2,4,6-trinitrotoluene, was added to a compost mix containing straw/horse manure, alfalfa, and horse feed. The temperature inside the pile reached 55°C. After 22 weeks, the total explosives content of the compost was reduced by 99% (Williams et al. 1989).

2,4,6-Trinitrotoluene has been reported to persist in groundwater for long periods of time by Rosenblatt (1980). However, other estimates of the half-life of the compound in groundwater range from 1 to 12 months, based on estimated unacclimated aqueous anaerobic and aerobic biodegradation (Howard et al. 1991).

5.3.2.3 Soil

Solid chunks of 2,4,6-trinitrotoluene buried in soil or exposed on the soil surface can persist for many years (Rosenblatt 1980). In smaller amounts, 2,4,6-trinitrotoluene may undergo photolysis in surface soils to trinitrobenzene and trinitrobenzaldehyde (Ryon et al. 1984).

5. POTENTIAL FOR HUMAN EXPOSURE

The transformation of 2,4,6-trinitrotoluene in soils has been found to be influenced by a number of environmental factors. In a study using ring-labeled ^{14}C-2,4,6-trinitrotoluene, the effects of soil organic matter content, 2,4,6-trinitrotoluene concentration, oxygen concentration, moisture content, temperature, incubation period, and microbial activity on 2,4,6-trinitrotoluene transformation in soil were examined. The soil pH was maintained at 6.5 throughout the test. In samples collected for analysis after 6 months and 11 months incubation, biological transformation was highest in soils containing the lowest concentration of 2,4,6-trinitrotoluene (0.1%) and lowest in soils containing the highest starting concentration of 2,4,6-trinitrotoluene (10%). The highest concentrations of degradation products were recovered from the soils receiving the lowest 2,4,6-trinitrotoluene loadings. Degradation products identified included 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, and diamines. As biotransformation increased, the amount of unextractable radioactive residue increased, suggesting that the metabolites of 2,4,6-trinitrotoluene exhibited stronger sorption to soils than the parent compound. Degradation decreased in sterilized soils. Of the environmental parameters evaluated in this study, the initial 2,4,6-trinitrotoluene concentration and the soil moisture level had the most influence on the rate of 2,4,6-trinitrotoluene transformation. The presence or absence of microbial activity and incubation temperature had less effect, and the remaining variables had no effect on 2,4,6trinitrotoluene transformation (Army 1985a).

The white rot fungus *Phanerochaete chrysosporium* was found to degrade ring labeled ^{14}C-2,4,6-trinitrotoluene sorbed to soils. After 30 days incubation, 6.3% of the sorbed 2,4,6-trinitrotoluene was recovered as $^{14}CO_2$. An additional 63.6% of the radioactivity was recovered in acetonitrile extracts, and 25.2% was unextractable. In the acetonitrile extract, only 2.2% of the radiolabel was in the form of undegraded 2,4,6-trinitrotoluene (Fernando et al. 1990).

In the same study, soil cultures containing 10,000-mg/kg loadings of ring-labeled ^{14}C-2,4,6-trinitrotoluene were extracted after 30-, 60-, and 90-day incubation periods and mass balances were calculated. The 90-day mass balance indicated that 18.4% of the radioactivity was recovered as $^{14}CO_2$, 62.6% in the form of metabolites present in the acetonitrile extract fraction, and 11.5% was bound to the soil/fungal matrix. The concentration of residual undegraded 2,4,6-trinitrotoluene in the 90-day acetonitrile extract was 14.9%, versus the >99% activity in the control samples (Fernando et al. 1990).

5. POTENTIAL FOR HUMAN EXPOSURE

In screening studies of 190 species of fungi from 98 genera, 183 species were found to be able to transform 2,4,6trinitrotoluene in 5-day shake culture tests. Transformation products included 4-amino-2,6-dinitrotoluene, 4-hydroxylamino-2,6-dinitrotoluene, and 4,4′-azoxy-2,2′,6,6′-tetranitrotoluene. None of the test organisms exhibited an ability to cleave the aromatic ring of 2,4,6-trinitrotoluene (Parrish 1977). A sulfate-reducing bacterium, *Desulfovibrio*, has been isolated that degrades 2,4,6-trinitrotoluene. However, this isolate also does not degrade 2,4,6-trinitrotoluene all the way to carbon dioxide (Boopathy and Kulpa 1992).

Composting of 2,4,6-trinitrotoluene in soils has been examined in laboratory scale and large-scale tests. In laboratory tests with ring labeled ^{14}C-2,4,6-trinitrotoluene, rapid biotransformation was found, with initial average activity levels of 93.5% reduced to 46.6% and 16.6% after 3 weeks and 6 weeks, respectively. No degradation products were detected in samples collected after 3 weeks. Minor amounts (i.e., less than 2%) were detected in samples collected at 6 weeks. The degradation products included 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2,6-diamino-4-nitrotoluene, and 2,2′6,6′-tetranitro-4,4′-azoxytoluene. The decrease in ^{14}C-2,4,6-trinitrotoluene concentration was found to be correlated with a reduction in the solvent extractable activity and a significant increase in the compost-bound radioactivity. This finding suggests that 2,4,6-trinitrotoluene was transformed into more polar metabolites. Similar results were found in the large scale greenhouse trials, where 2,4,6-trinitrotoluene concentrations decreased from 19,041 μg/g to less than 17 μg/g after 3 weeks. 2,4,6-Trinitrotoluene was not detected in leachate samples from fresh compost piles. After 3 weeks, the leachate was found to contain 6% of the radiolabel. The mechanism for the rapid transformation of 2,4,6-trinitrotoluene in these systems is unclear; however, there is no evidence to suggest that transformation proceeds via cleavage of the benzene ring (Isbister et al. 1984). In field studies of aerated static piles, the effects of temperature on cornposting were examined. Under thermophilic conditions, extractable 2,4,6-trinitrotoluene was reduced from 11,840 μg/g to 3 μg/g, while under mesophilic conditions, 2,4,6-trinitrotoluene was reduced from 11,190 μg/g to 50 μg/g (Williams et al. 1992).

Mixed microbial cultures isolated from two soils, one from a wooded area near a 2,4,6-trinitrotoluene loading facility and one from a greenhouse located 30 miles away, were tested for their ability to

degrade 2,4,6-trinitrotoluene. Both soils contained microbes capable of transforming 2,4,6-trinitrotoluene, added at a concentration of 100 mg/L, in the presence of yeast extract and glucose. Over the test period, 2,4,6-trinitrotoluene degradation was greater in the inoculum isolated from the soil near the 2,4,6-trinitrotoluene loading facility (36%) than for the remote soil (14%). Most of the microorganisms exhibiting 2,4,6-trinitrotoluene activity appeared to be pseudomonads (Osmon and Klausmeier 1973).

The estimated half-life of 2,4,6-trinitrotoluene in soils ranges from 1 to 6 months. This estimate was made on the basis of the estimated unacclimated aqueous aerobic biodegradation half-life (Howard et al. 1991). In laboratory tests with sandy loam and sandy silt loam soils, the aerobic degradation halflife of the compound was determined to be 5.7-7.7 days (EPA 1989c).

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.1 Air

Nitroaromatics associated with the manufacture and processing of military munitions have generally not been detected in atmospheric monitoring studies (EPA 1976a).

5.4.2 Water

2,4,6-Trinitrotoluene has been detected in surface water and groundwater samples collected only in the vicinity of munitions facilities. For example, the compound has been found in pink water effluents at concentrations of 774-998 ppb in lagoon water (Triegel et al. 1983) and 1-178 mg/L from LAP plants (Patterson et al. 1977).

2,4,6-Trinitrotoluene has been detected in groundwater samples collected in several monitoring studies conducted in the vicinity of munitions facilities. For example, using on-site high-performance liquid chromatography analysis, 2,4,6-trinitrotoluene was detected at concentrations of 320 μg/L at 200 feet downgradient and at 1 μg/L at 1,070 feet downgradient in groundwater samples collected at a demilitarization facility near Hawthorne, Nevada (Goerlitz and Franks 1989). As a result of leachates

from disposal of solid wastes in cesspools, burning areas, and on-site leaching pits, 2,4,6-trinitrotoluene was detected in groundwater samples collected at the Cornhusker Army Ammunition Plant near Grand Island, Nebraska (Spalding and Fulton 1988).

5.4.3 Soil

2,4,6-Trinitrotoluene has been detected in surface soil samples at an average concentration of 13,000 mg/kg at the U.S. Department of Energy's Weldon Spring site in St. Charles County, Missouri. The chemical plant at the site was used by the U.S. Army to produce 2,4,6-trinitrotoluene explosives in the 1940s (Haroun et al. 1990). At the West Virginia Ordnance Works located in Mason County, West Virginia, 2,4,6-trinitrotoluene and other nitroaromatics have been detected in surface soils at burning sites in concentrations of up to 4% (40,000 mg/kg). Nitroaromatics, principally 2,4,6-trinitrotoluene, were detected at up to 20,000 mg/kg within 5-10 meters of the foundations of processing and refining facilities (Kraus et al. 1985).

At the Lone Star Army Ammunition Plant located in Texarkana, Texas, 2,4,6-trinitrotoluene has been detected at a concentration of about 15% in samples of sludge taken from ponds used as solids settling areas for pink water effluent. 2,4,6-Trinitrotoluene concentrations were highest in surface soil samples (e.g., 18.8 mg/kg at 0.2-0.6 meter depth), with decreasing concentration with increased depth (e.g., <3 mg/kg below 4.5 meters) (Phung and Bulot 1981). Triegel et al. (1983) found 2,4,6-trinitrotoluene at concentrations of 200-56,700 ppm in sludge samples from pink water lagoons, and at 18.9-158 ppm in surface soil samples collected from directly beneath the lagoon.

5.4.4 Other Environmental Media

No information was found on the concentrations of 2,4,6-trinitrotoluene in other media.

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

According to the National Occupational Exposure Survey, a total of 31 workers were estimated to have been exposed to 2,4,6-trinitrotoluene in domestic workplaces in 1980 (NIOSH 1990).

5. POTENTIAL FOR HUMAN EXPOSURE

Workers involved in the production of munitions can be exposed to 2,4,6-trinitrotoluene during the manufacture of the compound and during its use to fill artillery shells, mines, and other explosive armaments. Historically, more workers have been involved in shell loading than in the manufacture of 2,4,6-trinitrotoluene. Workers are potentially exposed via inhalation of dust and vapors, and through dermal absorption of dust. Exposure to 2,4,6-trinitrotoluene vapors is possible when the compound is melted and poured into shells. Dust exposure is possible in tilling operations using 2,4,6-trinitrotoluene powder, loading of melt kettles, drilling for fuse placement, removal of excess solidified 2,4,6-trinitrotoluene from shells, and recycling excess 2,4,6-trinitrotoluene. Protection only against inhalation of dust or vapors may still result in potentially significant systemic exposure to 2,4,6-trinitrotoluene if skin exposure occurs, since dermal absorption is rapid and accounts for a significant portion of total exposure (Hathaway 1985).

A study by the U.S. Army Environmental Hygiene Agency examined 533 workers exposed to 2,4,6-trinitrotoluene in manufacturing and munitions processing operations. The 8-hour time-weightedaverage concentration of 2,4,6-trinitrotoluene ranged from less than 0.01 mg/m^3 to 1.84 mg/m^3; concentrations of greater than 0.5 mg/m3 were experienced by only 12.2% of the workers (Buck and Wilson 1975).

Diazo-positive metabolites and mutagenic activity of metabolites in the urine of workers have been used as indicators of exposure to nitro-aromatic and nitro-amino aromatic compounds, including 2,4,6-trinitrotoluene. In two studies conducted at a 2,4,6-trinitrotoluene manufacturing plant, urine samples were collected from groups of 32 and 50 individuals with variable exposure to 2,4,6-trinitrotoluene. Samples were collected at the end of a workshift and after a holiday or weekend. The workers were divided into three exposure categories: (1) no or low 2,4,6-trinitrotoluene exposure (e.g., laboratories, controlling departments, individuals with no 2,4,6-trinitrotoluene contact during the work shift preceding sampling); (2) medium 2,4,6-trinitrotoluene exposure (e.g., assembling grenades, octal-hexotol foundry, test foundry); and (3) high 2,4,6-trinitrotoluene exposure (e.g., trotyl foundry, sieve house). 2,4,6-Trinitrotoluene concentrations (vapor and dust) in breathing zone samples collected at workstations for each category were: (1) no detectable concentrations; (2) less than 0.3 mg/m^3; and (3) 0.3-0.5 or 0.6 mg/m^3. The concentration of diazo-positive metabolites and mutagenic metabolites in the urine samples was significantly higher in samples collected at the end of the work shift than in

samples collected after a holiday or a weekend for all three exposure categories, with the largest mean differences found in the highest exposure groups (Ahlborg et al. 1988a, 1988b).

Given the restricted production and use of 2,4,6-trinitrotoluene, exposure of members of the general population to the compound would probably be limited to populations living in the vicinity of hazardous waste sites or military munitions facilities. These individuals may be exposed to 2,4,6-trinitrotoluene through contact with contaminated environmental media, particularly groundwater. For example, using the multimedia screening model GEOTOX coupled with an exposure pathway model, McKone and Layton (1986b) identified consumption of contaminated water and ingestion of contaminated fruits and vegetables as the potentially most important exposure pathways for populations living near sites where 2,4,6-trinitrotoluene was released to surface soils. The investigation examined the relative importance of the following seven routes of exposure: (1) inhalation; (2) water consumption; (3) fruit and vegetable ingestion; (4) meat and dairy ingestion; (5) fish ingestion; (6) soil ingestion; and (7) dermal absorption. The least important of these pathways, according to the modeling exercise, were inhalation, soil ingestion, and dermal absorption.

As part of the baseline risk evaluation prepared for the remedial investigation of the U.S. Department of Energy's Weldon Spring Site located in Charles County, Missouri, dermal contact with and ingestion of contaminated surface soils, and inhalation of fugitive dust particulates were identified as the most important potential routes of exposure to 2,4,6-trinitrotoluene for workers involved in remedial actions at the site and for the general public. 2,4,6-Trinitrotoluene was detected in surface soils at the site in concentrations of up to 13,000 mg/kg. Modeling estimates of 2,4,6-trinitrotoluene concentrations in ambient air, resulting from the generation of fugitive dust, were $5x10^{-4}$ mg/m^3 and $1x10^{-4}$ mg/m^3 for total particulates and respirable particulates, respectively (Haroun et al. 1990).

Water quality criteria for the protection of human health from exposure to 2,4,6-trinitrotoluene of 44.25 μg/L (Dacre 1980) and 134.96 μg/L (Army 1987d) have been recommended; the latter value is based on a calculated acceptable daily intake of 0.28 mg/day.

5.6 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Workers involved in the manufacture of 2,4,6-trinitrotoluene and the processing of munitions containing the compound may be exposed to high concentrations of 2,4,6-trinitrotoluene in the workplace. Populations living near military munitions facilities or hazardous waste sites may also be exposed to high concentrations of 2,4,6-trinitrotoluene through contact with contaminated media.

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 2,4,6-trinitrotoluene 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 2,4,6-trinitrotoluene.

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 may be proposed.

5.7.1 Identification of Data Needs

Physical and Chemical Properties. The physical and chemical properties of 2,4,6-trinitrotoluene are sufficiently well defined to permit an assessment of the environmental fate of the compound to be made.

Production, Import/Export, Use, and Release and Disposal. On the basis of the limited production and specialized use of 2,4,6-trinitrotoluene, the compound is expected to have a fairly low human exposure potential. 2,4,6-Trinitrotoluene is not produced commercially in the United States; production of the compound is limited to military arsenals (HSDB 1990). Data on production volumes are not available because production is limited to military arsenals. In addition, import/export data are not available. The compound is a high explosive used by the military in the production of bombs and grenades. 2,4,6-Trinitrotoluene is released to the environment in liquid and solid wastes generated in the manufacture of the compound and the processing of munitions containing the compound. Media most likely to be contaminated include soils, surface water, and groundwater (Army 1986e). Wastes generated in the manufacture of 2,4,6-trinitrotoluene are characterized as hazardous wastes by EPA. Therefore, EPA regulations for their disposal must be followed. Additional studies of composting techniques in the treatment of 2,4,6-trinitrotoluene wastes would provide useful information in determining the effectiveness of these techniques in waste disposal.

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 1988, became available in May of 1990. This database will be updated yearly and should provide a list of industrial production facilities and emissions.

2,4,6-Trinitrotoluene is not subject to TRI reporting requirements. Therefore, release data for the compound are not available in TRI.

Environmental Fate. Upon release to the environment, 2,4,6-trinitrotoluene partitions to and is transported in surface water or groundwater. The compound is not expected to partition significantly to the atmosphere or to soils or sediment (HSDB 1990; Spanggord et al. 1985). 2,4,6-Trinitrotoluene undergoes rapid photolytic breakdown in surface waters to a number of degradation products. The compound also undergoes biotransformation in soils and surface water (Army 1985a; Fernando et al. 1990). Additional information on the persistence of 2,4,6-trinitrotoluene in surface water and groundwater, on the kinetics and characterization of the biodegradation products in soils and surface

waters, and on the identification of 2,4,6-trinitrotoluene complexes with humic materials would aid in determining the importance of these media with respect to potential human exposure. Since 2,4,6-trinitrotoluene has been found to persist in the soil following disposal, effort should be made to develop remediation technologies to minimize human exposure.

Bioavailability from Environmental Media. Available data from tests with laboratory animals and humans indicates that 2,4,6-trinitrotoluene is absorbed following dermal contact, ingestion, and inhalation (Army 1978a, 1981d; Hassman and Hassmanova 1976; Woollen et al. 1986). Although occupational exposure data indicate that the compound is absorbed via inhalation and dermal contact, little information is available regarding the absorption of 2,4,6-trinitrotoluene from contaminated environmental media. Additional information about uptake of the compound from contaminated environmental media, particularly following dermal contact with and ingestion of soils, would be helpful in identifying the most important routes of human exposure to 2,4,6-trinitrotoluene.

Food Chain Bioaccumulation. 2,4,6-Trinitrotoluene undergoes limited bioconcentration by plants and aquatic organisms (Liu et al. 1983b; Palazzo and Leggett 1986). As a result of its limited persistence in surface soils and surface waters and metabolism by terrestrial and aquatic organisms, the compound is not expected to biomagnify in terrestrial or aquatic food chains. Additional information on food chain bioaccumulation to confirm this predicted biomagnification behavior, particularly with respect to identification of 2,4,6-trinitrotoluene complexes with plant tissues, would be helpful in determining the relative importance of this route of exposure to humans.

Exposure Levels in Environmental Media. 2,4,6-Trinitrotoluene has been detected in soil, surface water, and groundwater samples taken at Army ammunition plants and at other military installations where the compound or munitions have been used or processed (Haroun et al. 1990; Kraus et al. 1985; Patterson et al. 1977; Spalding and Fulton 1988; Triegel et al. 1983). 2,4,6-Trinitrotoluene has not been detected in ambient air samples, in foods, or in ambient surface water or groundwater samples taken outside of military arsenals. The acceptable daily intake of the compound in drinking water has been estimated by the Army to be 0.28 mg/day. Additional information on the concentrations of 2,4,6-trinitrotoluene in environmental media at hazardous waste

sites and military arsenals, particularly in groundwater, would be helpful in estimating the doses that exposed populations may be receiving as a result of contact with these media.

Exposure Levels in Humans. Diazo-positive and mutagenically active metabolites of 2,4,6-trinitrotoluene have been detected in workers exposed to the compound at military facilities (Ahlborg et al. 1988a, 1988b). Additional information from biological monitoring of populations living in the vicinity of hazardous waste sites or military arsenals would aid in assessing the utility of biomarkers as indicators of human exposure.

Exposure Registries. No exposure registries for 2,4,6-trinitrotoluene were located. This substance is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for subregistries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to the exposure to this substance.

5.7.2 On-going Studies

A study is being conducted at the University of Illinois to examine the effect of the addition of organic amendments to soils contaminated with 2,4,6-trinitrotoluene. The study, which is sponsored by the U.S. Department of Agriculture, is evaluating the use of organic amendments to stabilize and bioremediate soils containing residual explosives.

At the Los Alamos National Laboratory, a study is being conducted on the uptake and biotransformation of explosives, including 2,4,6-trinitrotoluene, by plants.

Remedial investigations and feasibility studies at hazardous waste sites and military arsenals known to be contaminated with 2,4,6-trinitrotoluene should add to the available data base for environmental levels, environmental fate, and human exposure.

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring 2,4,6-trinitrotoluene 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 2,4,6-trinitrotoluene. 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 2,4,6-trinitrotoluene 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 are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Only limited data were located regarding methods of analysis for 2,4,6-trinitrotoluene and metabolites in biological samples. Methods have been developed to quantify these substances in blood, urine, tissues, and handswab samples. Details for selected methods are shown in Table 6-l.

The primary method that has been used to analyze for 2,4,6-trinitrotoluene and/or its metabolites in blood and urine is high-performance liquid chromatography (HPLC)/mass spectrometry (MS) (Yinon and Hwang 1985b, 1986b, 1986c, 1987). Blood samples are prepared for analysis by centrifuging to obtain the serum. The serum is extracted with methylene chloride and the solvent is exchanged to acetonitrile (Yinon and Hwang 1986b, 1987). A second method, in which plasma is extracted with toluene followed by solvent exchange to acetonitrile and HPLC/ultraviolet (UV) detection, had a detection limit in the ppb range, with good precision (10% relative standard deviation [RSD]) and accuracy (Army 1981b). Since no information on sensitivity or reliability was provided for the MSbased method, and no other methods for blood were located, the adequacy of the available HPLC

TABLE 6-1. Analytical Methods for Determining 2,4,6-Trinitrotoluene in Biological Materials

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Centrifuge sample; dilute serum with water; extract with methylene chloride; centrifuge and add sodium sulfate; filter and evaporate; redissolve in methylene chloride; evaporate and redissolve in acetonitrile	HPLC/MS	NR	NR	Yinon and Hwang 1986b
Urine	Acidify sample to hydrolyze; neutralize and extract with toluene; add sodium sulfate and filter; evaporate and redissolve in acetone or acetonitrile	HPLC/MS	0.1 µg/L	90% (TNT); 30% (metabolites)	Yinon and Hwang 1985b, 1986c
Urine	Acidify sample; neutralize and extract with toluene; add anhydrous sodium sulfate	GC/ECD	≈50 µg/L	90%	Almog et al. 1983
Urine	Acidify and heat sample; neutralize and extract with diethyl ether; evaporate and redissolve in acetone; develop silica gel plate with benzene/diethyl ether/methanol	TLC/densitometry	100 ng/spot	83–98%	Liu et al. 1991

TABLE 6-1. Analytical Methods for Determining 2,4,6-Trinitrotoluene in Biological Materials (continued)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plasma, kidney	Add sodium chloride/acetic acid solution to sample; extract with toluene; add water and evaporate organic phase; add acetonitrile containing internal standard; filter	HPLC/UV	248 µg/L (plasma); 211 ng/g (kidney)	86% (plasma); 75% (kidney)	Army 1981b
Muscle, fat	Homogenize sample; extract with acetonitrile; concentrate; add internal standard and water; filter	HPLC/UV	62 ng/g	87%	Army 1981b
Liver	Homogenize sample; add acetic acid/sodium chloride solution; extract with toluene; evaporate and redissolve in acetonitrile containing internal standard; filter	HPLC/UV	50 ng/g	49%	Army 1981b
Handswabs	Wipe hand with swab soaked in MTBE and extract with MTBE in pentane; centrifuge to remove debris; clean up on Amberlite® XAD-7 eluting with ethyl acetate	HRGC/ECD	1 ng/swab	78%	Douse 1985, 1987; Douse and Smith 1986

TABLE 6-1. Analytical Methods for Determining 2,4,6-Trinitrotoluene in Biological Materials (continued)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Handswabs (standards)	Wipe hand with dry swab; extract with methanol/potassium phosphate; directly inject standards	HPLC/PMDE	24 pg/inj	NR	Lloyd 1983a, 1991
Handswabs	Wipe hand with swab soaked in acetone; squeeze out acetone and concentrate	HPLC/TEA; HRGC/TEA	Low pg	NR	Fine et al. 1984
Handswabs	Swab hand with swab soaked in ethanol; extract in water/buffer solution with vortexing; add aliquots to antibody-coated micro-titer plates	ELISA	15 ng/swab	NR	Fetterolf et al. 1991

ECD = electron capture detection; ELISA = enzyme-linked immunosorbent assay; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; inj = injection; MS = mass spectrometry; MTBE = methyl-tert-butyl ether; NR = not reported; PMDE = pendant mercury drop electrode; TEA = thermal energy analyzer; TLC = thin-layer chromatography; TNT = 2,4,6-trinitrotoluene; UV = ultraviolet detection

methods for determining 2,4,6-trinitrotoluene and/or its metabolites in blood could not be completely evaluated. However, HPLC/MS is known to be a highly selective and sensitive method and results with urine samples indicate that it should also be a good method for determining 2,4,6-trinitrotoluene in blood. Hydrolyzed urine samples are extracted with toluene and the solvent is exchanged to acetone prior to separation by HPLC (Yinon and Hwang 1985b, 1986c, 1987). The limited data on this method of analyzing urine show a high recovery for 2,4,6-trinitrotoluene but much lower recovery for its metabolites. The high sensitivity (the limit of detection is in the sub-ppb range) and selectivity of the MS detector compensate for the low recovery of the metabolites. Gas chromatography (GC)/electron capture detection (ECD) and thin-layer chromatography (TLC)/densitometry have also been used to detect 2,4,6-trinitrotoluene and/or its metabolites in urine. GC/ECD accurately determined the 2,4,6-trinitrotoluene metabolite, 4-ADNT, in a toluene extract of hydrolyzed human urine (Almog et al. 1983). The limit of detection for this method was in the low-ppb range with high recovery of the analyte. A modification of TLC that employed a computer-linked densitometer for detection and quantitation reliably measured 2,4,6-trinitrotoluene and its metabolites in hydrolyzed human urine (Liu et al. 1991). Advantages of this method were its rapidity and low cost, However, it is about 3-4 orders of magnitude less sensitive than HPLC/MS and requires substantially more sample.

An HPLC/UV method has been developed for determining 2,4,6-trinitrotoluene in animal kidney, muscle/fat, and liver (Army 1981b). Detection limits for these matrices were in the low-to-mid ppb, and the analyses were reproducible with RSDs of 15% or better. There were some problems with recovery of the analytes, especially in liver samples. No other methods were available for comparison.

High-resolution gas chromatography (HRGC) with ECD or thermal energy analysis (TEA) and HPLC with electrochemical detection (EC) using a pendant mercury drop electrode (PMDE) or TEA have been proposed for the detection of 2,4,6-trinitrotoluene in handswabs (Douse 1985, 1987; Douse and Smith 1986; Fine et al. 1984; Lloyd 1983a, 1991). Limited data available indicate that both HPLC and HRGC are good separation methods for this analysis. Sensitivities for all three detectors are in the pg-to-low-ng range. TEA is slightly more selective for nitroaromatics, but the PDME has the advantage of being easily renewable which reduces contamination problems. An additional method based on monoclonal antibody technology was also located (Fetterolf et al. 1991). The enzyme-linked immunosorbent assay (ELISA) had a detection limit of about 15 ng/swab and showed no

cross-reactivity with other explosives or common contaminants. Electron spin resonance spectrometry has also been tested for handswab analysis and was found to be selective and specific (Bums et al. 1987). Sensitivity was comparable to other methods and precision was high (1.8% RSD).

6.2 ENVIRONMENTAL SAMPLES

Methods have been developed to detect 2,4,6-trinitrotoluene and some of its breakdown products in air, water, soil, plant tissue, explosives, explosives residues, and postblast debris. Methods include semiquantitative screening methods that can be used in the field and quantitative laboratory-based methods. Selected methods for analysis of environmental samples are presented in Table 6-2.

The primary method of analyzing for 2,4,6-trinitrotoluene in air is by GC, usually with ECD (Andersson et al. 1983; Bishop et al. 1981, 1988; Pella 1976, 1977; Van Slyke et al. 1985). Methods based on MS, including ion dilution MS (IDMS) (St. John et al. 1975) and glow discharge MS (GDMS) (McLuckey et al. 1988), have also been used successfully to measure 2,4,6-trinitrotoluene vapor in air. For most methods, the sample is collected in a tube containing a solid sorbent and desorbed with an organic solvent. A few methods have been developed that permit direct entry of the sample into the detecting instrument. These include GDMS (McLuckey et al. 1988) and ion mobility spectrometry (IMS) (Karasek and Denney 1974; Spangler et al. 1983). The latter can be adapted to a portable instrument for field use. A field method based on TLC has also been used (Chrostowski et al. 1976), but is more time consuming and much less sensitive than IMS. The limited data on sensitivity, accuracy, and precision make it difficult to compare these parameters for the different methods. However, most of the methods will detect 2,4,6-trinitrotoluene in air at the ppb level or less. While GC/ECD is the most commonly used method, several of the other methods have distinct advantages, such as increased sensitivity (GDMS and IMS), simplicity of sample collection/preparation (GDMS and IMS), or portability (IMS).

Methods have been developed to measure 2,4,6-trinitrotoluene and/or it breakdown products in drinking water, surface water, groundwater, waste water effluents, and sea water. The two methods most frequently used to analyze water for the presence of 2,4,6-trinitrotoluene and other polynitroaromatic hydrocarbons are HPLC/UV and HRGC/ECD. In addition, methods based on

TABLE 6-2. Analytical Methods for Determining 2,4,6-Trinitrotoluene in Environmental Samples

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect sample in glass wool-charcoal absorbent tubes; desorb with benzene	GC/ECD	<0.05 ppb	74–108%	Pella 1976
Air	Collect sample in Tenax®-GC or Florisil® adsorbent tubes; desorb with acetonitrile	GC/ECD	NR	96–101% (Tenax); 85–94% (Florisil)	Bishop et al. 1981
Air	Collect on Amberlite® XAD-2; desorb with toluene	GC/ECD	NR	77–87%	Andersson et al. 1983
Air	Collect sample in sampling bulb containing known amount of d_6-TNT in benzene; equilibrate for 1 hour; remove sample and wash bulb with benzene; evaporate in sample capillary	IDMS	≈0.1 ppb	NR	St. John et al. 1975
Air	Direct incorporation of sample into glow discharge chamber	GDMS	≈1.4 ppt	NR	McLuckey et al. 1988
Air	Collect sample on Chromosorb® 102 adsorbent column; desorb with acetone; visualize developed plate with diphenyl-amine in ethanol and expose to ultraviolet light	TLC	NR	NR	Chrostowski et al. 1976

TABLE 6-2. Analytical Methods for Determining 2,4,6-Trinitrotoluene in Environmental Samples (continued)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Direct incorporation of sample into reaction chamber	IMS	0.01 ppb	NR	Spangler et al. 1983
Incinerator emission	Collect sample on sampling train containing Amberlite® XAD-2 resin; desorb with toluene	GC/ECD	0.025 µg/mL	69–100%	Van Slyke et al. 1985
Water	Add internal standard to sample; collect sample on Amberlite® XAD-2 or XAD-4; dry column and extract with dichloromethane; dry on anhydrous Na_2SO_4; concentrate and exchange solvent to methanol	HRGC/ECD	NR	95%	Feltes et al. 1990
Water	Collect on SEP-PAK® cartridges and elute with methanol; concentrate; elute from reverse-phase column with methanol/ water	HPLC/UV	0.05–0.1 µg/L	29–79%	Army 1981a
Tap water, groundwater	Collect sample on Amberlite® XAD-4 resin; elute with ethyl acetate; concentrate	HRGC/ECD	<0.1 µg/L	95–97%	Richard and Junk 1986

TABLE 6-2. Analytical Methods for Determining 2,4,6-Trinitrotoluene in Environmental Samples (continued)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water, groundwater	Extract sample with toluene	HRGC/ECD	0.06 µg/L	102-105%	Hable et al. 1991
Waste water effluents	Add internal standard to sample; cleanup on SEP-PAK® cartridge, eluting with acetonitrile/water; centrifuge; elute from reverse-phase HPLC column with methanol/water	HPLC/UV	0.2 mg/L	75–95%	Army 1983b
Waste water, groundwater	Form membrane by dissolving polyvinyl chloride, deoctyl-phthalate, and polyoxyethylamine in tetrahydrofuran; react membrane with water sample in sealed chamber; remove membrane and analyze	Spectrophoto meter	10 µg/L	95–105%	Zhang et al. 1989
Waste water effluents, groundwater	Dilute sample with methanol/acetonitrile; filter; elute from reverse-phase column with methanol/aceto-nitrile/water	HPLC/UV	14 µg/L	101%	Army 1985b; Jenkins et al. 1986
Sea water	Add internal standard to sample; extract with benzene; evaporate; redissolve in benzene	GC/ECD	≈20 ng/L	70%	Hoffsommer and Rosen 1972

TABLE 6-2. Analytical Methods for Determining 2,4,6-Trinitrotoluene in Environmental Samples (continued)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Well water, surface water	Collect sample on Amberlite XAD-4® resin; rinse sorbent with distilled water and elute with acetone; concentrate; add methanol/water	HPLC/ED	2 µg/L	30–120%	Army 1986c
Surface water (brooks, ponds)	Collect sample on Amberlite XAD-2/4/8® resin; dry; desorb with dichloromethane; dry over anhydrous sodium sulfate; solvent exchange to methanol; concentrate; elute from reverse-phase column with methanol/water	HPLC/UV	50 ng/L	85–105%	Feltes and Levsen 1989
Surface water (lakes)	Extract with methylene chloride; cleanup on silica gel SEP-PAK® if needed; concentrate and exchange solvent to acetonitrile	HPLC/UV	6–11 µg/L	NR	Powell et al. 1983
Drinking water	Extract with toluene; inject into instrument	HRGC/ECD	NR	100–102%	Belkin et al. 1985

TABLE 6-2. Analytical Methods for Determining 2,4,6-Trinitrotoluene in Environmental Samples (continued)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Groundwater	Collect sample on Hayesep-R® solid sorbent cartridge; elute with acetone; concentrate; add internal standard; solvent exchange to methanol/water	HPLC/UV/PC	5–14 µg/L	62–82%	Army 1989b
Soil	Air dry, grind, homogenize sample; extract with acetonitrile in ultrasonic bath; dilute with aqueous $CaCl_2$; filter; elute from reverse-phase column with water/methanol	HPLC/UV	0.08 µg/g	102%	Jenkins et al. 1989
Soil	Extract with acetonitrile in ultrasonic bath; centrifuge and filter; elute from reverse-phase column with acetonitrile/water/methanol	HPLC/UV	0.1 µg/g	95–106%	Jenkins and Grant 1987
Soil	Air dry, grind; extract with acetonitrile in ultrasonic bath; add $CaCl_2$; filter	HPLC/UV	NR	70%	Bauer et al. 1990

TABLE 6-2. Analytical Methods for Determining 2,4,6-Trinitrotoluene in Environmental Samples (continued)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Stabilize soil samples at 20–30% moisture; homogenize; extract by sonication with acetonitrile; centrifuge and filter; elute from reverse-phase column with methanol/water	HPLC/UV	0.76 µg/g	99%	Bongiovanni et al. 1984
Soil	Air dry, grind, homogenize; extract with acetonitrile in ultrasonic bath; centrifuge and filter extract; elute from reverse-phase column with methanol/water	HPLC/UV	0.8 µg/g	98.2%	Army 1987c
Soil	Extract soil with methanol; filter extract; add calcium chloride and refilter; pump through indicator tube	Indicator tube	0.5 µg/g	58–70%	Army 1990b
Soil	Extract soil with acetone; filter; read background absorbance; add potassium hydroxide and sodium sulfite; filter; read absorbance and apply background correction	Spectrophoto meter	1.1 µg/g	63–96%	Army 1990b

TABLE 6-2. Analytical Methods for Determining 2,4,6-Trinitrotoluene in Environmental Samples (continued)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plant stems	Add sodium chloride and water to ground sample; extract with hexane/isopropanol; evaporate and add acetonitrile containing internal standard and water; filter	HPLC/UV	90 ng/g	52%	Army 1981b

$CaCl_2$ = calcium chloride; ECD = electron capture detection; ED = electrochemical detection; GC = gas chromatography; GDMS = glow-discharge mass spectrometry; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; IDMS = isotope-dilution mass spectrometry; IMS = ion-mobilization spectrometry; NA_2SO_4 = sodium sulfate; NR = not reported; PC = photoconductivity detector; TLC = thin-layer chromatography; d_6-TNT = fully deuterated TNT; UV = ultraviolet detection

colorimetry and spectrophotometry have been used to screen field samples for 2,4,6-trinitrotoluene and other polyaromatic hydrocarbons. A method involving direct injection of water samples into a mass spectrometer proved to be fast and simple, but the high detection limit makes it useful only for screening (Yinon and Laschever 1982). In addition, MS is expensive and requires more technical training than some of the other methods used for analysis of 2,4,6-trinitrotoluene in water.
With the HPLC- and GC-based methods, sample preparation usually involves direct solvent extraction of the sample (Army 1985b; Bauer et al. 1986; Belkin et al. 1985; Hable et al. 1991; Hoffsommer and Rosen 1972; Jenkins et al. 1986; Leggett et al. 1990; Maskarinec et al. 1984; Powell et al. 1983) or concentration on a solid sorbent (Army 1981a, 1983b; Feltes et al. 1989, 1990; Maskarinec et al. 1984; Richard and Junk 1986).

HPLC/UV is the method usually employed by the Army to measure 2,4,6-trinitrotoluene in waste water effluents from munitions plants (Army 1981a, 1983b, 1985b; Bauer et al. 1986; Jenkins et al. 1986; Leggett et al. 1990). HPLC methods are relatively simple and fast, accurate, and selective. In explosives analysis, where many of the analytes are thermally-labile, they also have the advantage of not requiring heat. Sensitivity is generally in the low-to-mid ppb range. Substitution of a photodiode array detector (PAD) for the usual ultraviolet detector allowed detection of ppt in water (Feltes and Levsen 1989). HPLC with EC at a gold-mercury electrode increased sensitivity relative to UV and also improved selectivity (Maskarinec et al. 1984).

HRGC/ECD is also a sensitive and selective method for determination of 2,4,6-trinitrotoluene in water. Detection limits in the sub-ppb range are obtainable and recoveries are high (Belkin et al. 1985; Feltes et al. 1990; Hable et al. 1991; Hoffsommer and Rosen 1972; Richard and Junk 1986). In addition, precision is excellent with the RSD usually less than 10%. Detection by TEA or MS has also been used with GC with good results (Feltes et al. 1990). A comparison of these detection methods with ECD showed that while TEA was more selective than ECD for nitro compounds and had a larger linear concentration range, ECD was more sensitive than TEA by about three orders of magnitude (Feltes et al. 1990). MS was the most selective of the three detection methods, and was determined to be useful for confirmatory analysis. One disadvantage of GC methods in explosives analysis is that thermally-labile analytes may be destroyed during analysis.

6. ANALYTICAL METHODS

The available optical methods rely on the conversion of 2,4,6-trinitrotoluene to a fluorescent or colored complex (Army 1990b; Heller et al. 1977, 1982; Jian and Seitz 1990; Zhang et al. 1989). In general, these methods are not as sensitive or selective as the more commonly used HPLC and GC methods and they are primarily useful for simple and rapid screening of samples at field sites to determine which samples should be subjected to more intensive quantitative analysis. A recently proposed spectrophotometric method reacts a polynitroaromatic hydrocarbon-contaminated water sample with a membrane containing polyoxyethylamine. The reaction produces a colored product that can be analyzed with a spectrophotometer (Zhang et al. 1989). Since different polynitroaromatic hydrocarbons produce different absorption spectra, the method is selective as well as relatively sensitive (detection of low ppb). The method is used to screen samples in the field and, by employing fiber optics, may be useful for remote monitoring (Zhang and Seitz 1989).

An HPLC/UV method developed by the Army is the method most commonly used to analyze soils and sediments for 2,4,6-trinitrotoluene and its breakdown products (Army 1985c, 1987c; Bauer et al. 1990; Bongiovanni et al. 1984; Jenkins and Grant 1987; Jenkins et al. 1989). Sample extraction consists of homogenizing the soil or sediment, extraction with an organic solvent, and separation on a reverse-phase HPLC column. The method is sensitive and reliable and has been used to determine 2,4,6-trinitrotoluene and some of its metabolites at levels in the low ppb range. GC/ECD has also been used to analyze soil samples for 2,4,6-trinitrotoluene (Army 1985c). Sample preparation is similar to that used for HPLC. Results from GC analysis were mixed, with some samples producing results comparable to HPLC, but others (those samples high in organic matter) subject to substantial interference. More recent efforts by the Army have focused on the development of simple, rapid methods that can be used to screen samples in the field. Two semiquantitative methods have been tested, one based on an indicator tube originally designed for testing water samples (Army 1990b; Heller 1982) and the other based on spectrophotometry (Army 1990a). Both methods could detect 2,4,6-trinitrotoluene in the sub- to low-ppm range but were not specific for 2,4,6-trinitrotoluene. The indicator tube was inexpensive, simple, rapid, and easy to use. However, it was found to have poor accuracy and precision. The spectrophotometric method had better accuracy and precision and estimated concentrations correlated well with laboratory analyses of the same samples. It is not as convenient as the indicator tube, however, because a battery-operated spectrophotometer must also be carried into the field.

A method for determining 2,4,6-trinitrotoluene in plant stems using HPLC/UV was located (Army 1981b). The limit of detection for the method was in the ppb, but both recovery (52%) and precision (33% RDS) were poor. No other methods in similar matrices were available for comparison,

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 2,4,6-trinitrotoluene 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 2,4,6-trinitrotoluene.

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 may be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Only a few methods for monitoring exposure to 2,4,6-trinitrotoluene were located. These included HPLC/MS, GC/ECD, and TLC/densitometry for determining 2,4,6-trinitrotoluene or its metabolites in blood and/or urine (Almog et al. 1983; Liu et al. 1991; Yinon and Hwang 1985b, 1986c) and HPLC/UV for determining 2,4,6-trinitrotoluene in tissues (Army 1981). In addition, there are methods for analyzing for the chemical and its metabolites in handswab samples (Douse 1987; Fetterolf et al. 1991; Fine et al. 1984; Lloyd 1991). While these methods appear to be useful and reliable for measuring 2,4,6-trinitrotoluene in biological samples, the limited information on sensitivity, selectivity, accuracy, and precision make

it difficult to compare and fully evaluate the reliability of the methods. More information is needed on these parameters for the different methods in order to assess their usefulness for monitoring exposure to 2,4,6-trinitrotoluene.

Possible biomarkers of effect for 2,4,6-trinitrotoluene exposure include changes in hematological and blood chemistry parameters, such as decreased hemoglobin and hematocrit levels, increased mononuclear leukocyte and lymphocyte counts, and changes in levels of SGOT and LDH (Army 1976, 1978a; Morton et al. 1976). Reliable standard clinical laboratory methods exist to measure these parameters; however, they are not specific to 2,4,6-trinitrotoluene exposure and have only limited use as biomarkers of effect for this chemical. Urine discoloration and cataracts are also observed in workers exposed to 2,4,6-trinitrotoluene, but no methods are available to quantitate these nonspecific biomarkers.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods exist to detect and quantify 2,4,6-trinitrotoluene in air, fresh water, sea water, waste-water effluents, soil, and plant material (Army 1981, 1985b; 1986c, 1988, 1990a, 1990b; Bauer et al. 1990; Feltes and Levsen 1989; Feltes et al. 1990; Hable et al. 1991; Hoffsommer and Rosen 1972; Jenkins et al. 1989; McLuckey et al. 1988; Spangler et al. 1983; Van Slyke et al. 1985; Zhang et al. 1989). The HPLC- and GC-based methods are generally considered to be sensitive and reliable, but in some cases (e.g., air samples) better characterization is needed. Some of the newer methods (e.g., IMS, GDMS) and those proposed for field use (e.g., IMS, membrane-based spectrophotometry) need continued research and characterization in order to be useful quantitative methods for analysis of environmental samples (Army 1990b; McLuckey et al. 1988; Spangler et al. 1983; Zhang et al. 1989).

6.3.2 On-going Studies

No on-going analytical methods studies were located. However, it is likely that research is continuing on some of the more recently introduced methods for analyzing biological and environmental samples,

such as the use of ELISA (Fetterolf et al. 1991). GDMS (McLuckey et al. 1988), IMS (Spangler et al. 1983), and spectrophotometric field methods (Army 1990b; Zhang et al. 1989).

7. REGULATIONS AND ADVISORIES

The international, national, and state regulations and guidelines regarding 2,4,6-trinitrotoluene in air, water, and other media are summarized in Table 7- 1.

ATSDR has derived an MRL of 0.0005 mg/kg/day for intermediate oral exposure to 2,4,6-trinitrotoluene (see Section 2.4). EPA (IRIS 1994) assigned 2,4,6-trinitrotoluene a reference dose (RfD) of $5.00x10^{-4}$ mg/kg/day with an uncertainty factor of 1,000 based on liver effects observed in dogs in a 26-week feeding study (Army 1983a).

EPA has assigned 2,4,6-trinitrotoluene a weight-of-evidence carcinogenic classification of C, which indicates that 2,4,6-trinitrotoluene is a possible human carcinogen (IRIS 1994).

The Drinking Water Equivalent Level (DWEL), a lifetime exposure at which adverse health effects would not be expected to occur, is 20 μg/L for 2,4,6-trinitrotoluene (EPA 1989b, 1994). Because of the lack of appropriate data for determination of the One-day Health Advisory and the Ten-day Health Advisory, it is suggested that the DWEL be used as a conservative estimate. The Longer-term Health Advisory for both children and adults is 20 μg/L, which is equivalent to the DWEL. The Lifetime Health Advisory is 2 μg/L.

Based on EPA Guidelines, an acceptable daily intake of 44.25 μg/L was calculated (Army 1987d). The available data for calculating water quality criteria for 2,4,6-trinitrotoluene were insufficient to meet all of the EPA guidelines requirements. However, enough information was available to calculate a reasonable estimate of the criterion maximum concentration, 557 μg/L, to protect aquatic life. The other component of the criteria, the criterion continuous concentration, needs further research. The criterion to protect human health has been estimated to be 135 μg/L, but further research is needed to confirm this value.

TABLE 7-1. Regulations and Guidelines Applicable to 2,4,6-Trinitrotoluene

Agency	Description	Information	References
NATIONAL			
Regulations:			
a. Air:			
OSHA	PEL TWA (skin designation)	0.5 mg/m^3	OSHA 1989a (29 CFR 1910.1000); OSHA 1989b
b. Other:			
EPA OSW	Listing as a hazardous waste: Wastewater treatment sludge from the manufacturing and processing of explosives	Yes	EPA 1989c
DOT	Designated as a hazardous substance subject to requirements for packaging, labeling, and transportation	Yes	DOT 1989a (49 CFR 172.101); Appendix A); DOT 1989b
	TNT dry--class A explosive (high explosive)		
	TNT wet--flammable solid		
Guidelines:			
a. Air:			
ACGIH	TLV TWA (skin designation)	0.5 mg/m^3	ACGIH 1993
NIOSH	REL TWA (10 hours)	0.5 mg/m^3	NIOSH 1990
ACGIH	STEL	No data	ACGIH 1993
b. Water			
EPA ODW	Health advisories		EPA 1993
	DWEL	20 µg/L	
	One-day	20 µg/L	
	Ten-day	20 µg/L	
	Longer term	20 µg/L	
	Lifetime	2 µg/L	
	10^{-4} Cancer risk level	100 µg/L	
	MCL	No data	
	MCLG	No data	
c. Other:			
EPA			
	RfD (oral)	5.0×10^{-4} mg/kg/day	EPA 1993
	Carcinogenic classification	Group C*	EPA 1993
	Unit risk (air)	No data	IRIS 1994
	Unit risk (water)	No data	IRIS 1994
STATE			
Regulations and Guidelines:			
a. Air:	Acceptable Ambient Air Concentrations		NATICH 1992
Connecticut	(8 hours)	1.00×10^{1} µg/m^3	
Florida-Pinellas	(8 hours)	5.00 µg/m^3	
Florida-Pinellas	(24 hours)	1.20 µg/m^3	
Nevada	(8 hours)	1.20×10^{-2} mg/m^3	
North Dakota	(8 hours)	5.00×10^{-3} mg/m^3	
Oklahoma	(24 hours)	5.00×10^{1} µg/m^3	
Texas	(30 minutes)	5.00 µg/m^3	
Texas	(Annual)	5.00×10^{-1} µg/m^3	
Virginia	(24 hours)	8.30 µg/m^3	

7. REGULATIONS AND ADVISORIES

TABLE 7-1. Regulations and Guidelines Applicable to 2,4,6-Trinitrotoluene (continued)

Agency	Description	Information	References
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		CELDS 1991
Alabama		Yes	
Alaska		Yes	
Arizona		Yes	
California		Yes	
Connecticut		Yes	
Colorado		Yes	
Delaware		Yes	
Florida		Yes	
Georgia		Yes	
Hawaii		Yes	
Idaho		Yes	
Indiana		Yes	
Iowa		Yes	
Louisiana		Yes	
Maryland		Yes	
Massachusetts		Yes	
Michigan		Yes	
Minnesota		Yes	
Mississippi		Yes	
Missouri		Yes	
Montana		Yes	
Nebraska		Yes	
New Jersey		Yes	
New Mexico		Yes	
North Carolina		Yes	
North Dakota		Yes	
New York		Yes	
Ohio		Yes	
Oregon		Yes	
Rhode Island		Yes	
South Dakota		Yes	
Tennessee		Yes	
Texas		Yes	
Utah		Yes	
Virginia		Yes	
Vermont		Yes	
Washington		Yes	
Washington, D.C.		Yes	
West Virginia		Yes	
Wisconsin		Yes	
Wyoming		Yes	
	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.		CELDS 1991
Alabama	Pretreatment standards for discharge	Yes	
	Hazardous waste: thermal treatment	Yes	
Arizona	Solid waste collection	Yes	

TABLE 7-1. Regulations and Guidelines Applicable to 2,4,6-Trinitrotoluene (continued)

Agency	Description	Information	References
Arkansas	Solid waste storage and collection	Yes	
Colorado	Fugitive dust	Yes	
Connecticut	Hazardous waste: thermal treatment	Yes	
Florida	Hazardous waste: thermal treatment	Yes	
Georgia	Open burning	Yes	
Illinois	Sewer discharge	Yes	
Indiana	Open burning	Yes	
Kentucky	Hazardous waste management	Yes	
Louisiana	Open burning	Yes	
Minnesota	Hazardous waste management	Yes	
New Hampshire	Open burning	Yes	
New Jersey	Hazardous waste management	Yes	
New Mexico	Hazardous waste management	Yes	
Nevada	Hazardous waste management	Yes	
North Carolina	Hazardous waste: thermal treatment	Yes	
North Dakota	Fugitive emissions	Yes	
Pennsylvania	Fugitive emissions	Yes	
South Carolina	Open burning	Yes	
Tennessee	Hazardous waste: thermal treatment	Yes	
Utah	Hazardous waste management	Yes	
Vermont	Open burning	Yes	
Virginia	Solid waste management	Yes	
Wisconsin	Open burning and malodorous emissions	Yes	
	Explosive control laws regulate storage, manufacture, and use (regulations vary from state to state)		CELDS 1991
Alaska		Yes	
California		Yes	
Connecticut		Yes	
Georgia		Yes	
Hawaii		Yes	
Indiana		Yes	
Iowa		Yes	
Kansas		Yes	
New Jersey		Yes	
Massachusetts		Yes	
Nebraska		Yes	
New Jersey		Yes	
Oklahoma		Yes	
Oregon		Yes	
Washington, D.C.		Yes	
West Virginia		Yes	
Wisconsin		Yes	

[a]Group C=Possible human carcinogen

ACGIH = American Conference of Governmental Industrial Hygienists; DOT = Department of Transportation; DWEL = Drinking Water Equivalent Level; EPA = Environmental Protection Agency; NIOSH = National Institute for Occupational Safety and Health; ODW = Office of Drinking Water; OSHA = Occupational Safety and Health Administration; OSW = Office of Solid Waste; PEL = Permissible Exposure Limit; REL = Recommended Exposure Limits; RfD = Reference Dose; TLV = Threshold Limit Value; TNT = 2,4,6-Trinitrotoluene; TWA = Time-Weighted Average

Under the Hazardous Materials Transportation Act, 2,4,6-trinitrotoluene is designated as a hazardous substance subject to special requirements for packaging, labeling, and transportation as a result of its explosive/flammable properties (DOT 1989a, 1989b).

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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.

9. GLOSSARY

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

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

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

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.

9. GLOSSARY

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

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

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 $\mu g/m^3$ for air).

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

Reportable Quantity (RQ) -- The quantity of a hazardous substance that is considered reportable under CERCLA. Reportable quantities are (1) 1 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-l and 2-2) are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, minimal risk levels (MRLs) to humans for noncancer 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-l and Figure 2-l are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 2-l

(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 lS), 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., Nits&e et al. 1981.

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

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

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

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

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

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

LEGEND

See Figure 2-1

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

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

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

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

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

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

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

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

SAMPLE

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

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
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

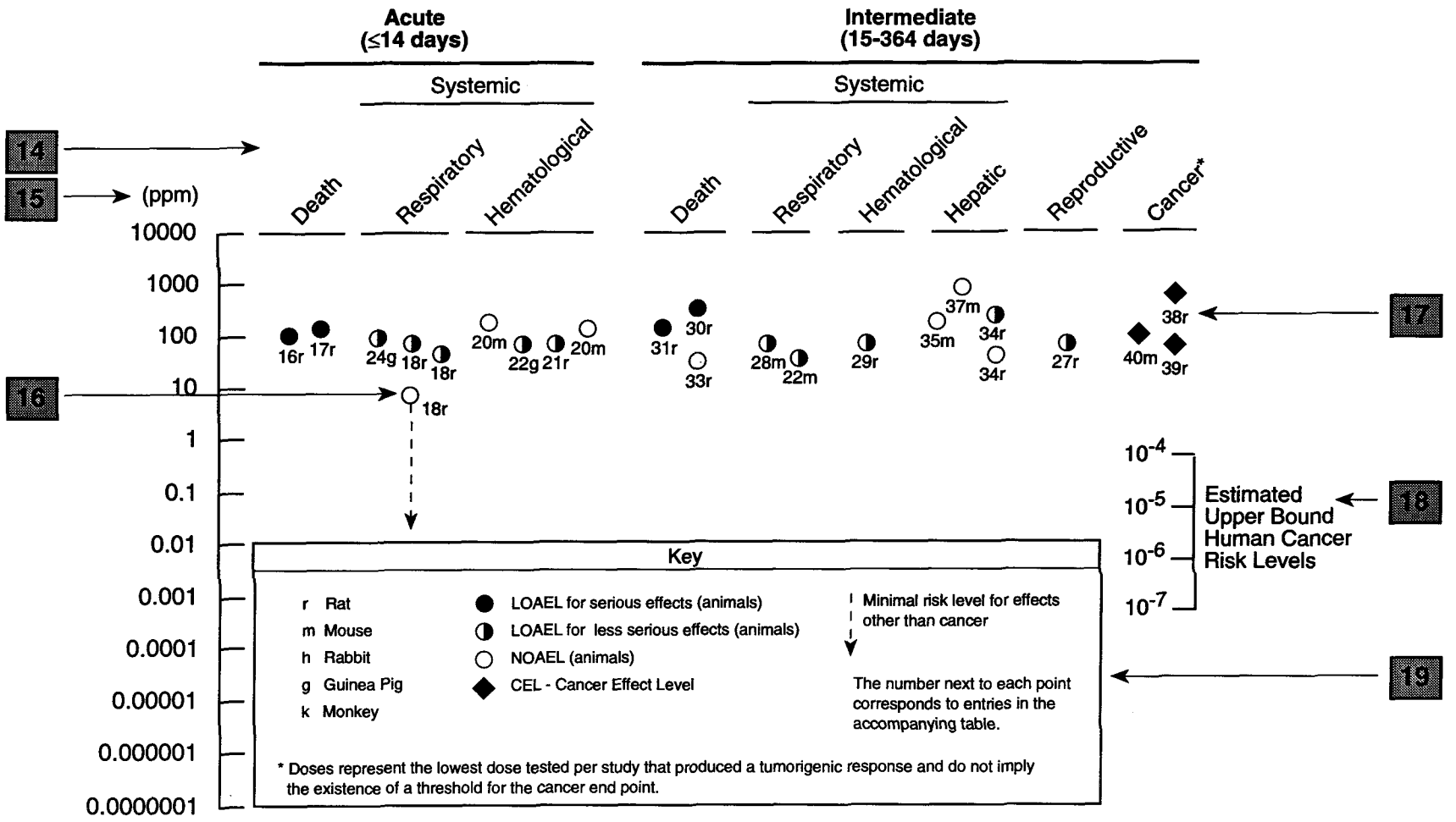
[a] The number corresponds to entries in Figure 2-1.

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.

1. What effects are known to occur in humans?
2. What effects observed in animals are likely to be of concern to humans?
3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The section covers 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 (RfDs).

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 r&at& 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
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
mmHg	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
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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