

**DRAFT
TOXICOLOGICAL PROFILE FOR
DINITROTOLUENES**

**PUBLIC COMMENT PERIOD ENDS:
September 30, 2013**

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

April 2013

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UPDATE STATEMENT

A Toxicological Profile for 2,4- and 2,6-Dinitrotoluene was released in 1998. This present edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

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FOREWORD

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

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

- (A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;
- (B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and
- (C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public. We plan to revise these documents in response to public comments and as additional data become available. Therefore, we encourage comments that will make the toxicological profile series of the greatest use.

Electronic comments may be submitted via: www.regulations.gov.
Follow the on-line instructions for submitting comments.

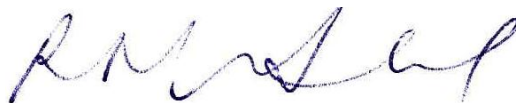
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The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "...effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the National Priorities List, in an effort to "...establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

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



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QUICK REFERENCE FOR HEALTH CARE PROVIDERS

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

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Relevance to Public Health: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by type of health effect (death, systemic, immunologic, reproductive), by route of exposure, and by length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.

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

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

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

Other Sections of Interest:

Section 3.8	Biomarkers of Exposure and Effect
Section 3.11	Methods for Reducing Toxic Effects

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY) **Fax:** (770) 488-4178
E-mail: cdcinfo@cdc.gov **Internet:** <http://www.atsdr.cdc.gov>

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

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

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

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

Other Agencies and Organizations

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

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

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

Referrals

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: <http://www.aoec.org/>.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.
2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.
3. Data Needs Review. The Environmental Toxicology Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.
4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

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PEER REVIEW

A peer review panel was assembled for dinitrotoluenes. The panel consisted of the following members:

1. William J. George, Ph.D., Department of Pharmacology SL83, Tulane University School of Medicine, New Orleans, Louisiana;
2. James Klaunig, Ph.D., Professor and Director of Toxicology, Department of Pharmacology and Toxicology, Division of Toxicology, School of Medicine, Indiana University, Indianapolis, Indiana;
3. Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin (BGFA), Ruhr-Universität Bochum, Bürkle-de-la-Camp Platz 1, 44789, Bochum, Germany.

These experts collectively have knowledge of dinitrotoluene'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.

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 FOR DINITROTOLUENES

Overview

We define a public health statement and show how it can help you learn about dinitrotoluenes (DNTs).

Introduction

A public health statement summarizes information about a hazardous substance. The information is taken from a toxicological profile developed by the Agency for Toxic Substances and Disease Registry's (ATSDR's) Division of Toxicology and Human Health Sciences (DTHHS). A toxicological profile is a thorough review of a hazardous substance.

This toxicological profile examines DNTs. This public health statement summarizes the DTHHS's findings on DNTs, describes the effects of exposure to them, and describes what you can do to limit that exposure.

DNTs at hazardous waste sites

The U.S. Environmental Protection Agency (U.S. EPA) identifies the most serious hazardous waste sites in the nation. U.S. EPA then includes these sites the National Priorities List (NPL) and targets them for federal clean-up activities. U.S. EPA has found DNT in at least 98 of the 1,699 current or former NPL sites.

The total number of NPL sites evaluated for DNT is not known. However, the possibility remains that as more sites are evaluated, the number of sites at which DNT is found may increase. This information is important; these future sites may be sources of exposure, and exposure to DNT may be harmful.

Why a DNT release can be harmful

When a contaminant 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. However, such a release doesn't always lead to exposure. You can only be exposed to a contaminant when you come in contact with it. That contact—and therefore that exposure—can occur when you breathe, eat, or drink the contaminant, or when it touches your skin.

Even if you're exposed to DNTs, you might not be harmed. Whether you are harmed will depend on such factors as the dose (how much), the duration (how long), and how you happen to contact it. Harm might also depend on whether you've been exposed to any other chemicals, as well as your age, sex, diet, family traits, lifestyle, and state of health.

1. PUBLIC HEALTH STATEMENT FOR DINITROTOLUENES

A Closer Look at DNTs

Overview

This section describes DNTs in detail and how you can be exposed to them.

What is DNT? DNT is comprised of a mixture of isomers. These particular isomers have the same molecular weight and molecular formula and the same organic functional groups. However, the organic functional groups are at different positions of the benzene ring. Two of the isomers of DNT, 2,4-DNT and 2,6-DNT, make up 95% of DNT. The other 5% is predominantly comprised of four other isomers (2,3-, 2,5-, 3,4-, and 3,5-DNT). DNT is not a natural substance and is commercially produced by reacting concentrated sulfuric and nitric acid with toluene.

How are DNTs used? DNT is a substance produced during the conversion of toluene to toluene diisocyanate (TDI), a precursor to polyurethane polymers. It is also used to make trinitrotoluene (TNT).

Where are DNTs found? DNT can be released into the air, water, and soil at places where it is produced or used. It is not commonly found outside of source areas or, in other words, its manufacturing facilities or contaminated waste sites.

Possible Sources	Outcome
Air: DNT is rarely detected in ambient air, but is detected in workplace air where it is manufactured or used.	Occupational exposure is possible, but DNT inhalation exposure to the general population is very low.
Water: Military and industrial activities have lead to reported release of DNT into soil, groundwater or surface water. DNT levels as high as 10,000 µg/L were reported in potable groundwater at the Joliet Army Ammunition Plant located in Illinois.	DNT is slowly broken down in water by microbial organisms and it can be broken down by sunlight in surface water.
Soil: DNT has been detected in soil at levels of ~100 mg/kg at areas like ammunition sites and military firing ranges.	DNT does not adsorb strongly to soil. Therefore, it can move from soil into groundwater, where it can contaminate drinking water.
Consumer Products: DNT is not used extensively in consumer products and is not often detected in food samples.	

1. PUBLIC HEALTH STATEMENT FOR DINITROTOLUENES

How DNT Can Affect Your Health

Overview

This section looks at how DNTs enter your body and potential DNT health effects found in human and animal studies.

How DNTs enter your body

DNTs can enter your body from the air or water.

Possible Sources	Possible Exposure Pathway
Air	If you breathe air containing DNT, it will enter your body through your lungs.
Water	If present in drinking water, DNT will rapidly enter your body through the digestive tract.
Soil	Some soil samples may contain a high level of DNT. You can be exposed dermally if you come into contact with soil contaminated with DNT.

How DNTs leave your body

DNTs leave your body rapidly. They break down into other chemicals that leave your body in the urine within 24 hours. Small amounts of the DNTs may also be present in the feces.

Introduction to DNT health effects

The health effects of DNTs depend on how much DNT you are exposed to and the length of that exposure. Environmental monitoring data are limited, but they do suggest that any DNT levels the public might encounter by contact through air, water, or soil are generally much lower than animal-study levels.

Short-term exposure effects

Animal studies show that ingestion of DNTs can cause anemia, and damage to the nervous system, male reproductive system, and liver. Animal studies have shown that breathing vapors or aerosols of DNTs can cause damage to the lungs. Breathing or ingesting very high levels of DNTs may cause death.

Long-term exposure effects

A study using workers reported a relationship between heart disease and long-term exposure to DNT. Animal studies have shown that ingesting DNTs over long periods causes anemia, and damage to the nervous system, male reproductive system, and liver. Ingestion of DNTs over long periods may also cause death.

1. PUBLIC HEALTH STATEMENT FOR DINITROTOLUENES

DNTs and cancer Several studies in workers have looked for an association between DNT exposure and cancer. No increases in the risk of liver or kidney cancer were found. Two studies did find an increased risk of urothelial cancers in workers. Studies using workers have not indicated whether DNTs cause cancer. Laboratory animals ingesting DNTs during most of their lives developed cancer of the liver and tumors in the kidneys.

Some cancer findings by government and other agencies

- The U.S. EPA says a mixture of 2,4- and 2,6-DNT is a probable human carcinogen, based on findings of cancer in animal studies.
- The International Agency for Research on Cancer says 2,4- and 2,6-DNT are possibly carcinogenic to humans, but that carcinogenicity for 3,5-DNTs in humans cannot be determined due to a lack of information.

See Chapters 2 and 3 for more information on health effects of DNTs.

Children and DNTs

Overview

This section discusses potential health effects of DNT exposure in humans from when they're first conceived to 18 years of age, and how you might protect against such effects.

Exposure effects for children generally No data describe the effects of exposure to DNTs on children or young animals. Although we think that children would likely show the same health effects as adults, we don't know whether children are more susceptible than adults to DNT effects.

What about birth defects? We don't know whether DNTs can harm an unborn child. Results of animal studies show that newborns of mothers exposed to DNTs during pregnancy can have anemia and nervous system damage at birth. These effects are similar to those seen in adult animals.

Drinking water Limit exposure to contaminated drinking water. DNT is not often detected in drinking water supplies.

Medical Tests to Determine DNT Exposure

Overview

We identify medical tests that can detect whether DNTs are in your body, and we recommend safe toxic-substance practices.

1. PUBLIC HEALTH STATEMENT FOR DINITROTOLUENES

DNTs can be measured in urine

DNT and its breakdown products (metabolites) can be measured in urine. However, the detection of DNTs or metabolites cannot predict the kind of health effects that might develop from that exposure. Because DNTs and their metabolites leave the body fairly rapidly, the tests need to be conducted within days after exposure.

For more information on the different substances formed by DNT breakdown and on tests to detect these substances in the body, see Chapters 3 and 7.

Federal Government Recommendations to Protect Human Health

Overview

One way the federal government promotes public health is by regulating toxic substances or recommending ways to handle or to avoid toxic substances.

The federal government regulates toxic substances

Regulations are enforceable by law. The U.S. EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that have adopted toxic substances regulations.

The federal government recommends safe toxic substance practices

ATSDR and the National Institute for Occupational Safety and Health (NIOSH) have made recommendations about toxic substances. Unlike enforceable regulations, these recommendations are advisory only.

Toxic substance regulations

Regulations and recommendations can be expressed as “not-to-exceed” levels (that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value usually based on levels that affect animals); levels are then adjusted to help protect humans. These not-to-exceed levels sometimes differ among federal organizations. Different organizations use different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or emphasize some factors over others, depending on their mission.

Check for regulation updates

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that issued the regulation or recommendation.

1. PUBLIC HEALTH STATEMENT FOR DINITROTOLUENES

Some regulations and recommendations for DNTs include

Federal Organization	Regulation or Recommendation
U.S. Environmental Protection Agency (U.S. EPA)	The U.S. EPA has determined that exposure to 2,4-DNT in drinking water at concentrations of 1 mg/L for 1 or 10 days is not expected to cause any adverse effects in a child.
Occupational Safety and Health Administration (OSHA)	OSHA set a legal limit of 1.5 mg/m ³ DNT in workplace air averaged over an 8-hour work day.
National Institute for Occupational Safety and Health (NIOSH)	NIOSH recommends a limit of 1.5 mg/m ³ DNT in workplace air averaged over a 10-hour work day.

Additional Information

Overview

Where to find more information about DNTs.

Whom to contact first

If you have any more questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

Additional information from ATSDR

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

Where to obtain toxicological profile copies

Toxicological profiles are also available online at www.atsdr.cdc.gov and on CD-ROM. Request a copy of the ATSDR ToxProfiles™ CD-ROM by:

- Calling the toll-free information and technical assistance number at 1-800-CDCINFO (1-800-232-4636),
- E-mailing cdcinfo@cdc.gov, or
- Writing to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Human Health Sciences
1600 Clifton Road NE
Mailstop F-57
Atlanta, GA 30333
Fax: 1-770-488-4178

1. PUBLIC HEALTH STATEMENT FOR DINITROTOLUENES

For-profit organizations should request final toxicological profile copies from:

National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000
Web site: <http://www.ntis.gov/>

1. PUBLIC HEALTH STATEMENT FOR DINITROTOLUENES

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2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO DNT IN THE UNITED STATES

Dinitrotoluene (DNT) is generally produced as a mixture called technical-grade DNT (Tg-DNT), which contains approximately 76.5% 2,4-DNT and 18.8% 2,6-DNT (with the remainder consisting of other isomers and minor contaminants such as TNT and mononitrotoluenes). It is primarily used as a chemical intermediate for the production of toluene diisocyanate. DNT is also used in the production of 2,4,6-TNT, dyes, and polyurethane foams. 2,4-DNT has reportedly been used in air bags of automobiles.

When released to the environment, DNT has the potential to leach into groundwater or contaminate surface waters through soil runoff and erosion. Volatilization from water bodies or soil surfaces occurs slowly for DNT and as a consequence, it is not frequently detected in ambient air. DNT generally biodegrades slowly under environmental conditions, but it is rapidly degraded by photolysis in natural water. Measured bioconcentration factors in fish suggest that the potential for bioaccumulation is low in fish and other aquatic organisms.

DNT is very infrequently detected in drinking water. There were no detections of 2,4-DNT in 3,251 samples taken from small public water systems and there was only 1 detection out of 30,513 samples obtained from large systems throughout the United States. 2,6-DNT was not detected in any of the 33,765 samples (both large and small systems) for which it was tested. DNT has been detected in surface water and groundwater near source locations such as munitions sites. Concentrations of 2,4- and 2,6-DNT obtained from a small brook and a river adjacent to a former ammunition plant were 0.5–13.0 µg/L and 0.1–7.6 µg/L, respectively. DNT levels as high as 10,000 µg/L were reported in potable groundwater at the Joliet Army Ammunition Plant located in Will County, Illinois. 2,3-, 2,5-, 3,4-, and 3,5-DNT isomers were identified in both monitoring wells and a few private water supply wells near an Army ammunition site in Wisconsin. 2,4-DNT was measured in soil samples obtained from the Joliet Army Ammunition at levels of <0.1 mg/kg (detection limit) to 117 mg/kg. 2,6-DNT was detected on this site at concentrations ranging from <0.1 to 8 mg/kg.

The major route of exposure to DNT for populations residing near hazardous waste sites or munitions facilities is via ingestion of contaminated water or dermal contact with contaminated soil. Dermal exposure to DNT could also occur when washing or bathing with contaminated water. Occupational exposure by inhalation or dermal contact may occur at workplaces where DNT is manufactured or used.

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Monitoring data suggest that populations that do not live near source areas are not exposed to significant levels of DNT.

2.2 SUMMARY OF HEALTH EFFECTS

Data on health effects of DNT are available from occupational exposure studies and studies in laboratory animals. Most occupational exposure studies evaluate health effects in workers exposed to 2,4-, 2,6-, or technical-grade (Tg)-DNT. However, most of these studies were conducted before 1950 and provide very little information on exposure concentrations. Furthermore, interpretation of study results is limited due to the absence of appropriate control groups, mixed exposures to other chemicals, and small number of workers studied. Nearly all studies in laboratory animals are oral exposure studies of 2,4-, 2,6-, or Tg-DNT, and include data for acute, intermediate, and chronic exposure durations. Very little information is available regarding health effects of inhaled DNT; however, systemic effects of DNT are expected to be similar for all routes of exposure.

Results of occupational exposure studies and studies in laboratory animals identify the hematological (methemoglobinemia, anemia, and compensatory hematopoiesis) and nervous systems (clinical signs of neurotoxicity, ataxia, tremors, leg weakness, and convulsions) as the most sensitive targets of DNT-induced toxicity. In addition to hematological and neurological effects, results of animal studies provide evidence that the liver, respiratory tract, and reproductive system also are targets for DNT-induced toxicity. However, effects to these other organ systems occur at levels that are higher than those producing hematological effects or neurotoxicity under similar exposure conditions.

In occupational exposure studies, findings of anemia and cyanosis in workers exposed to 2,4- or Tg-DNT are consistent with the DNT-induced hematological effects observed in laboratory animals; however, available human data provide only limited evidence, as studies did not include adequate control groups or report exposure concentrations. Adverse hematological effects, specifically methemoglobinemia and anemia, have been reported in laboratory animals exposed to oral 2,4-, 2,6-, or Tg-DNT for acute, intermediate, and chronic durations. Hematological effects of DNT are initiated by methemoglobin production, which occurs when the ferrous iron in complex with the heme groups of hemoglobin is oxidized to ferric iron. Ferric iron does not bind oxygen and, therefore, reduces the oxygen-carrying capacity of the blood. Ferric iron also contributes to the denaturation of hemoglobin and subsequent removal of erythrocytes from the blood, resulting in anemia. Heinz bodies, granules of denatured hemoglobin, form and can be detected in erythrocytes. Increased hematopoiesis is often observed as a

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compensatory response to decreased erythrocyte count. Hematological parameters typically affected by DNT exposure include increased blood methemoglobin levels and reticulocyte count, decreased erythrocyte count, hematocrit, and blood hemoglobin levels, and the presence of Heinz bodies; the severity of effects increases with dose. In addition, extramedullary erythropoiesis of the spleen, a compensatory response, may also occur. Results of a single-dose oral exposure study in rats indicate that the onset of hematological effects is rapid, with effects observed 24 hours after administration of 99 mg/kg of 2,4- or 2,6-DNT. For acute exposure durations, the lowest daily oral dose associated with hematological effects is 4 mg/kg/day in dogs exposed to 2,6-DNT for 14 consecutive days. Similar hematological effects have been observed following intermediate-duration exposure, with the development of methemoglobinemia, anemia, and compensatory erythropoiesis in dogs administered daily oral doses of 1.5 mg/kg of 2,4-DNT for 3–9 months. Extramedullary erythropoiesis of the spleen was observed in dogs administered ≥ 4 mg/kg/day of 2,6-DNT for 4–13 weeks. For chronic-duration exposure, hematological effects were observed following exposure of dogs to 2,4-DNT at 1.5 mg/kg/day for 24 months. Following cessation of exposure, hematological effects showed complete reversal, although time to reversal increased with severity of effects.

In occupational exposure studies, neurological effects, including headache, dizziness, insomnia, unpleasant taste in the mouth, and pain, numbness, and tingling in the extremities, also have been reported in workers exposed to 2,4- or Tg-DNT; however, available human data provide only limited evidence, as studies did not include adequate control groups or report exposure concentrations. The nervous system also is identified as a sensitive target for DNT-induced toxicity, with symptoms of neurotoxicity reported in laboratory animals exposed to oral 2,4-, 2,6-, and Tg-DNT for acute, intermediate, and chronic durations. However, results of these studies indicate that, in general, the nervous system is a less sensitive target than the hematological system. Symptoms of neurotoxicity observed in laboratory animals include weakness, stiffness, or rigid paralysis of the hind legs, abnormal gait, tremors, ataxia, and convulsions, with severity increasing with dose. For acute- and intermediate-duration exposures, the lowest doses producing symptoms of neurotoxicity are 25 mg/kg/day in dogs exposed to 2,4-DNT for 12 days to 13 weeks and 20 mg/kg/day in dogs exposed to 2,6-DNT for 13 weeks. Demyelination in the cerebellum and brain stem was observed in rats exposed to dietary exposures of 93 mg/kg/day 2,4-DNT for 13 weeks. In chronic-duration exposure studies, the lowest dose producing neurotoxicity was 1.5 mg/kg/day 2,4-DNT in a 2-year study in dogs; however, this effect (loss of hindquarter control) was only observed intermittently in one of six dogs. More severe signs of neurotoxicity and central nervous system lesions (vacuolization, hypertrophy, endothelial mitosis, focal gliosis in the cerebellum, and perivascular hemorrhage in the cerebellum and brain stem) occurred at 10 mg/kg/day.

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Occupational exposure studies do not provide conclusive evidence of DNT-induced hepatotoxicity due to lack of appropriate controls. Hepatic effects have been observed in animals exposed to 2,4-, 2,6-, or Tg-DNT in laboratory animals. However, as discussed below, 2,4-, 2,6-, and Tg-DNT have been shown to induce hepatocellular carcinoma following chronic-duration oral exposure. Thus, hepatic effects observed at less-than-chronic exposure durations may represent early stages of progressive development to hepatic cancer. The potential for DNT to induce liver cancer complicates interpretation of study results showing possible effects in the liver, as hepatotoxicity may be a precursor to or a result from the development of hepatic neoplasms. Hepatic effects, including liver discoloration, inflammation, degeneration of hepatocytes, proliferation of bile duct epithelium, and elevated blood levels of hepatic enzymes, have been reported in oral exposure studies of 2,4-, 2,6-, or Tg-DNT in laboratory animals. Hepatic effects occurred at 2,4-DNT doses ≥ 10 mg/kg/day and at 2,6-DNT doses ≥ 7 mg/kg/day, with severity related to dose and exposure duration. At higher oral doses (≥ 50 mg/kg/day), onset of hepatic toxicity appears to be rapid; congested sinusoids with sloughed hepatocytes, infiltration of segmented neutrophils, pyknotic nuclei, microvesiculated cytoplasm, and apoptosis of hepatocytes were observed 48 hours after administration of 2,6-DNT. Following chronic dietary exposure to 2,4-DNT for 1 year, hepatocellular alterations were observed in male rats at a dose of 0.6 mg/kg/day; however, neoplastic nodules of the liver were also observed in these animals at doses ≥ 0.6 mg/kg/day. Biliary hyperplasia was observed in dogs exposed to 2,4-DNT for 24 months, with no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) values of 1.5 and 10 mg/kg/day, respectively.

Adverse reproductive effects, including effects on male and female reproductive systems and decreased neonatal viability, have been observed in laboratory animals following acute, intermediate, and chronic oral exposure to 2,4-, 2,6-, or Tg-DNT. Severity of reproductive effects is dose- and exposure duration-related, and the male reproductive system is more sensitive than the female reproductive system or neonatal viability. In males, adverse effects include decreased sperm production, testicular atrophy, degeneration of seminiferous tubules, and changes in Sertoli cell morphology. The lowest dose producing effects to the male reproductive system (testicular atrophy and decreased spermatogenesis) was 14 mg/kg/day in mice fed diets containing 2,4-DNT for up to 2 years. Effects on the female reproductive system (ovarian atrophy and nonfunctional follicles) were observed in mice fed 898 mg/kg/day 2,4-DNT. Decreased neonatal viability was observed in rats fed ≥ 34.5 mg/kg/day 2,4-DNT for up to 6 months in a three-generation reproductive study. Occupational exposure studies do not provide evidence of DNT-induced adverse reproductive effects due to both concomitant exposure of workers to other

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chemicals and reporting insufficiencies. Dietary exposure of rats to 2,4-DNT for up to 6 months in a three-generation reproductive study produced delayed eye opening at doses fed ≥ 35 mg/kg/day, but no other developmental effects. In neonates exposed to Tg-DNT during gestation, systemic effects similar to those observed in adult animals, including delayed eye opening and signs of mild neurological effects (cliff avoidance behavior) and effects on hematological parameters, were observed at maternal doses ≥ 100 mg/kg/day; recovery from these effects was observed by postpartum day 60. Although no studies were located regarding developmental effects in humans after oral exposure to DNT, developmental toxicity from DNT could potentially occur because exposure to any substance that depletes the amount of oxygen available to developing fetal tissues may cause toxicity to the developing organism.

Respiratory distress, pulmonary congestion, and increased relative lung weights were observed in rats following a single 6-hour inhalation exposure of rats to near-lethal or lethal concentrations of 2,6-DNT (vapor or aerosol). No effects on the respiratory system have been observed following acute, intermediate, or chronic oral exposure of laboratory animals to DNT. Occupational studies do not include reports of respiratory effects in workers.

The carcinogenic activity of DNT has been extensively studied in typical chronic bioassays and in some less-than-lifetime studies. Results show the development of renal cancer in mice fed 2,4-DNT at 95 mg/kg/day and hepatocellular carcinoma in male and female rats fed 2,4-DNT at doses of 34.5 and 45.3 mg/kg/day, respectively. Hepatocellular carcinomas were observed in rats administered 2,6-DNT at doses ≥ 7 mg/kg/day. 2,4-DNT was a hepatic tumor promoter, but not a tumor initiator, using *in vivo* hepatic initiation-promotion protocols. Both tumor-initiating and -promoting activities of 2,6- and Tg-DNT in rat liver were reported. A retrospective cohort mortality study at two army ammunition plants that used Tg-DNT and/or 2,4-DNT showed no significant increases in mortality from malignant neoplasms as a whole or from particular cancers (liver, lung, gallbladder, kidney, and connective tissues), although interpretation of study results is limited by a small cohort size. Two studies of copper miners exposed to high levels of Tg-DNT from explosives found significant increases in the incidence of urothelial cancers. An increase in renal cancers was also found; however, the increased incidence did not appear to be related to Tg-DNT exposure levels. The National Toxicology Program (NTP) has not evaluated DNT in its 12th Report on Carcinogens. EPA has classified 2,4-DNT/2,6-DNT mixture as a probable human carcinogen based on sufficient evidence of carcinogenicity in animals. The International Agency for Research on Cancer (IARC) has classified 2,4- and 2,6-DNT as possibly carcinogenic to humans, and 3,5-DNT as not classifiable as to its carcinogenicity to humans.

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Health effects of 2,4- and 2,6-DNT ingestion in laboratory animals and the dose ranges at which these effects occur are shown in [Figures 2-1](#) and [2-2](#), respectively. Estimates of oral doses posing minimal risk to humans (MRLs) are also presented in these figures.

2.3 MINIMAL RISK LEVELS (MRLs)

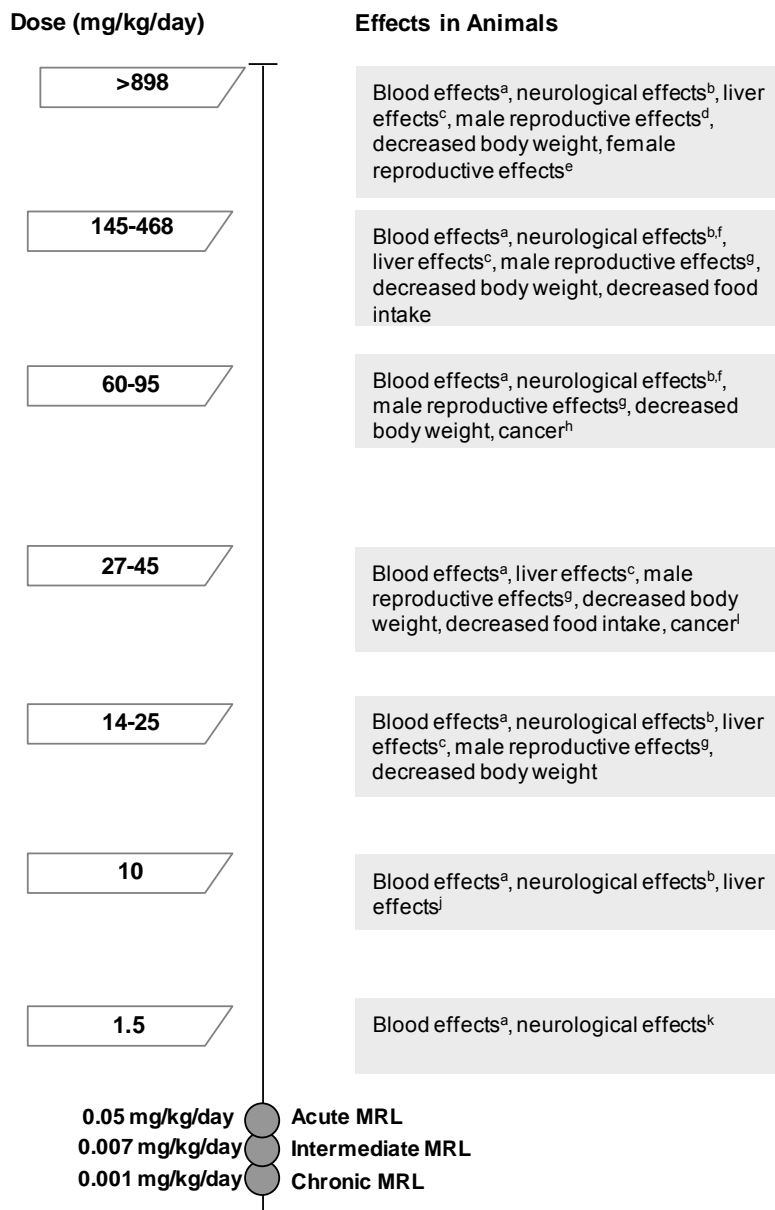
Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for 2,4- and 2,6-DNT. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs 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.

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 as appropriate.

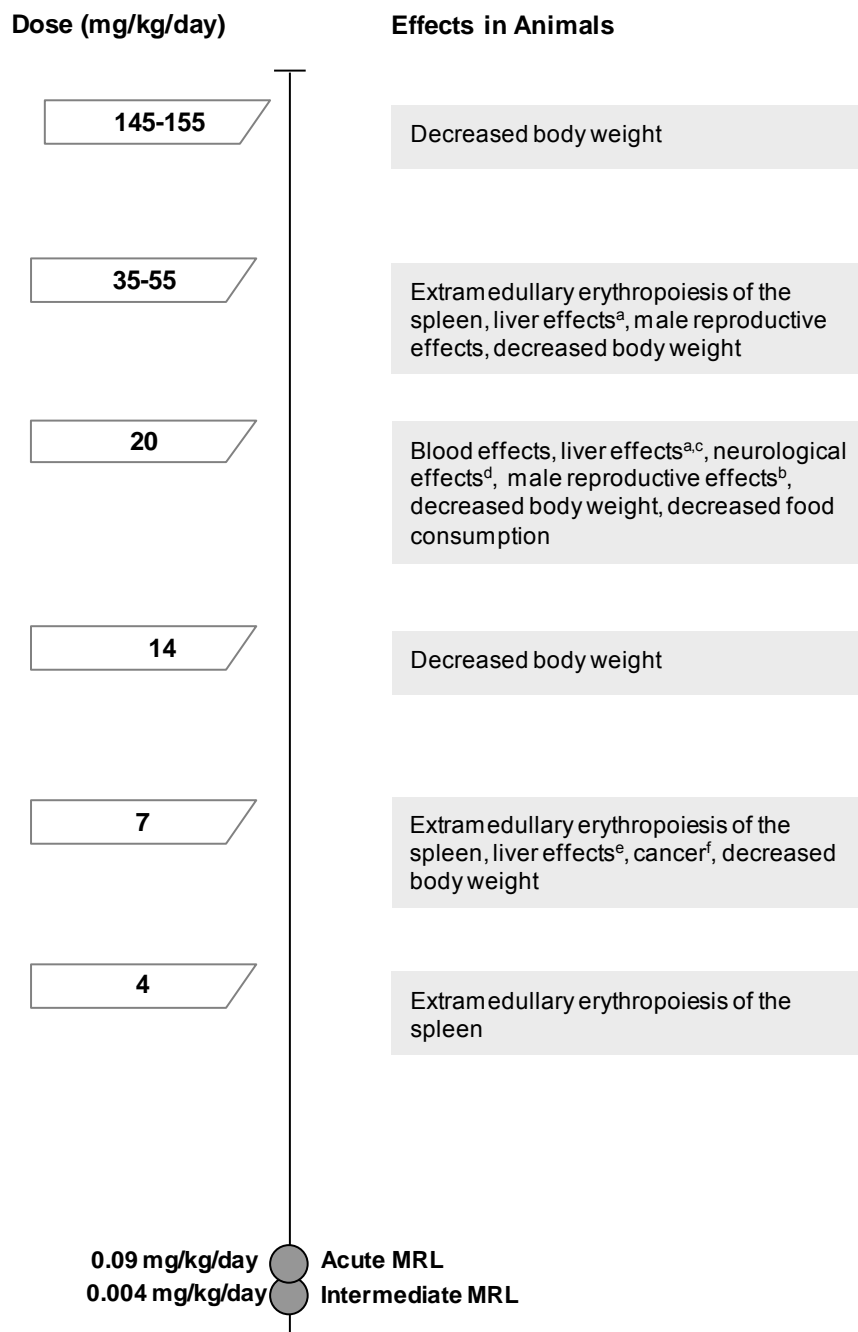
Inhalation MRLs

Most of the data on health effects associated with inhalation exposure of humans to DNT are from studies of workers exposed to 2,4- 2,6-, or Tg-DNT. However, most of these studies were conducted before 1950 and provide very little information on exposure concentrations. Furthermore, interpretation of study results is limited due to the absence of appropriate control groups, mixed exposures to other chemicals, and small number of workers studied. Therefore, while available occupational studies provide supportive information for observations in animals regarding identification of target organs, they do not report dose-response data that can be used to derive MRLs for DNT. Regarding available animal data, one study was located that examined the acute inhalation toxicity of 2,6-DNT in rats (CMA 1991). This study identified mortality and clinical signs of respiratory toxicity (exaggerated respiratory movements) in rats exposed to

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Figure 2-1. Health Effects for Ingesting 2,4-DNT^aMethemoglobinemia, anemia and compensatory hematopoiesis^bSigns of neurotoxicity (e.g., loss of hindquarter control, convulsions, incoordination, stiffness, abnormal gait, paralysis)^cHepatocellular degeneration or dysplasia^dDecreased fertility^eOvarian atrophy and nonfunctioning follicles^fHistopathologic changes to the central nervous system^gTesticular degeneration or atrophy and decreased or absent spermatogenesis^hRenal carcinomaⁱHepatocellular carcinoma^jBiliary hyperplasia^kIntermittent clinical signs of neurotoxicity (e.g., loss of hindquarter and convulsions)

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Figure 2-2. Health Effects for Ingesting 2,6-DNT^aBile duct hyperplasia^bTesticular degeneration, decreased spermatogenesis^cInflammatory changes^dIncoordination and lack of balance^eHepatocellular degeneration^fHepatocellular carcinoma

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2,6-DNT as an aerosol at concentrations ≥ 196 mg/m³; rats that died showed increased relative lung weights and evidence of lung congestion. No effects were observed in rats exposed nose-only to 2,6-DNT as a vapor at 26 mg/m³ for 6 hours and observed 14 days after dosing. No studies were located regarding inhalation exposure to 2,3-, 2,5-, or 3,5-DNT in experimental animals. Thus, data were insufficient for the derivation of acute-, intermediate-, and chronic-duration inhalation MRLs for 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT.

Oral MRLs

Acute-, intermediate-, and chronic-duration oral MRLs were derived for 2,4-DNT and acute- and intermediate-duration oral MRLs were derived for 2,6-DNT. For 2,4- and 2,6-DNT, studies in humans did not provide sufficient data regarding exposure levels and their correlation with observed effects; thus, animal studies were used for the derivation of these oral MRLs. Separate acute- and intermediate-duration MRLs were derived for 2,4- and 2,6-DNT because very little comparative data for these isomers are available. For 2,3- and 2,5-DNT, the only available oral exposure study is a single-dose study, which reported data on lethality; however, no additional information was reported (Vernot et al. 1977). No studies on the effects of oral exposure to 3,5-DNT in animals were identified. Therefore, data were insufficient for the derivation of acute-, intermediate-, and chronic-duration oral MRLs for 2,3-, 2,5-, and 3,5-DNT.

Acute-Duration Oral MRL for 2,4-DNT

- An MRL of 0.05 mg/kg/day has been derived for acute-duration oral exposure (≤ 14 days) to 2,4-DNT.

No human acute-duration oral toxicity studies on 2,4-DNT were identified. Acute-duration studies in animals identified the nervous system, hematological system, liver, and reproductive system as targets for DNT-induced toxicity, with neurotoxicity as the most sensitive effect. Neurotoxicity was observed following acute-duration oral exposure of beagle dogs, as part of an intermediate-duration study (U.S. Army 1978b). Dogs (4/sex/group) exposed to 2,4-DNT at 0, 1, 5, or 25 mg/kg/day in capsules for 13 weeks were observed for behavioral changes and clinical signs of toxicity during the first 14 days of treatment. Signs of neurotoxicity were evident at 25 mg/kg/day. Onset of neurotoxicity (loss of hind leg control) was observed in one female dog on day 12; three male dogs developed similar effects on day 14. Signs of neurotoxicity were reported in all dogs exposed to 25 mg/kg/day after treatment for 12–22 days. No clinical signs were observed in dogs treated with 1 or 5 mg/kg/day. Results of this study identify

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acute-duration NOAEL and LOAEL values for neurotoxicity in dogs of 5 and 25 mg/kg/day, respectively. Although comprehensive end points were not evaluated at 14 days, data obtained following 13 weeks of treatment in this study identify the same NOAEL and LOAEL values of 5 and 25 mg/kg/day, respectively, for neurotoxicity, hematological effects, and reproductive effects. Thus, it is unlikely that hematological or reproductive effects would occur at lower doses than neurotoxicity for acute-duration exposure.

Acute-duration studies in rodents identified the hematological system, liver, and reproductive system as targets of acute-duration exposure to 2,4-DNT. The most sensitive hematological effect (slight cyanosis) was observed in male Sprague-Dawley rats exposed to 2,4-DNT at 60 mg/kg/day (lowest tested dose) for 5 days (Lane et al. 1985). The lowest LOAELs for hepatotoxicity (increased cholesterol accompanied by increased serum alanine aminotransferase levels in males) were reported in Sprague-Dawley rats treated at 78 and 82 mg/kg/day (for males and females, respectively) for 14 days; NOAELs were not identified (McGown et al. 1983). The lowest LOAEL for reproductive effects (decreased thickness of spermatogenic cell layers) was 78 mg/kg/day for male Sprague-Dawley rats administered 2,4-DNT for 14 days (McGown et al. 1983). Lane et al. (1985) identified a NOAEL of 60 mg/kg/day, with a LOAEL of 180 mg/kg/day, for decreased fertility in male rats treated with 2,4-DNT for 5 days.

Neurotoxicity (loss of hind limb control) was identified as the most sensitive effect of acute-duration oral exposure to 2,4-DNT, based on the NOAEL value of 5 mg/kg/day in dogs administered 2,4-DNT via capsules for 12–14 days (U.S. Army 1978b). Based on the available data, neurotoxicity was selected as the critical effect for the basis of the acute-duration oral MRL. The NOAEL value of 5 mg/kg/day was used as the point of departure (POD). Neurotoxicity data were not suitable for benchmark dose (BMD) modeling, since effects were observed only at the highest dose tested. The POD of 5 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability), resulting in an acute-duration oral MRL for 2,4-DNT of 0.05 mg/kg/day.

Intermediate-Duration Oral MRL for 2,4-DNT

- An MRL of 0.007 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to 2,4-DNT.

Information on effects of intermediate-duration oral exposure of laboratory animals is available from studies treating animals with 2,4-DNT for intermediate durations (15–364 days) and from chronic-duration studies evaluating end points at intermediate-duration time points. Results of studies in dogs,

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rats, and mice show the development hematological, neurological, and reproductive effects following intermediate-duration exposure to 2,4-DNT, with the hematological effects as the most sensitive end point. Adverse hematological effects were observed in studies in dogs, rats, and mice, with the lowest NOAEL and LOAEL values of 1.5 and 10 mg/kg/day, respectively, observed following intermediate-duration oral exposure of dogs to 2,4-DNT (Ellis et al. 1985; U.S. Army 1979). As part of this 2-year study in dogs, hematological end points were evaluated in dogs administered 2,4-DNT at 3, 6, and 9 months as part of a 2-year study in dogs administered 0, 0.2, 1.5, or 10 mg/kg/day. Hematological effects consistent with development of methemoglobinemia were observed at doses of 1.5 and 10 mg/kg/day at all intermediate-duration time points (3, 6, and 9 months). Although effects at all time points were qualitatively similar, hematological changes observed after 9 months of exposure were more consistent and pronounced than those observed at the 3- and 6-month time periods (data are presented in Table A-1 of Appendix A). Male and female dogs exposed for 9 months at doses of 1.5 and 10 mg/kg/day showed detectable amounts of methemoglobin in the serum, with changes reaching statistical significance in males and females at 10 mg/kg/day. In female dogs administered 10 mg/kg/day, statistically significant decreases in erythrocyte count, hematocrit, and hemoglobin, a statistically significant increase in reticulocyte count, and the presence of Heinz bodies in serum were observed. Similar hematological effects were observed in female dogs administered 0.2 and 1.5 mg/kg/day, although effects did not reach statistical significance, most likely because the power of the study to detect statistically significant changes was compromised by the small number of dogs per treatment group. However, based on a clinically significant increase in methemoglobin levels of 225% in female dogs administered 1.5 mg/kg/day, the NOAEL and LOAEL values for hematological effects in this study are 0.2 and 1.5 mg/kg/day, respectively. Similar effects were observed in male dogs, although changes did not reach statistical significance in the 10 mg/kg/day group, possibly due to the small number of male dogs evaluated. Comprehensive end points were not evaluated at intermediate-duration time points in this study. However, the chronic-duration portion of this study, in which comprehensive end points were examined, identified hematological effects as the most sensitive effect of chronic-duration exposure. Thus, it is unlikely that effects to other target organs would occur at lower doses than the hematological effects for intermediate exposure durations (see overview of chronic-duration oral data).

Intermediate-duration studies in animals administered higher daily doses show the development of similar hematological effects, along with neurotoxicity and toxicity to the reproductive system. Beagle dogs administered 2,4-DNT for up to 13 weeks developed hematological effects (methemoglobinemia, anemia, and compensatory hematopoiesis), neurological effects (incoordination, abnormal gait, and paralysis), and reproductive effects (testicular degeneration and decreased spermatogenesis) at 25 mg/kg/day, with a

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NOAEL for these effects of 5 mg/kg/day (Lee et al. 1985; U.S. Army 1979). Hematological effects similar to those observed in dogs were reported in rats treated with 2,4-DNT at 93–371 mg/kg/day (Kozuka et al. 1979; U.S. Army 1978b) and in mice treated with 2,4-DNT at 413 and 468 mg/kg/day (for males and females, respectively) for up to 13 weeks (Hong et al. 1985; U.S. Army 1978b). The lowest LOAEL for neurological effects (clinical signs of neurotoxicity and demyelination of the cerebellum and brain stem) in rodents was 93 mg/kg/day for male CD rats administered 2,4-DNT for 4 or 13 weeks; the NOAEL for neurological effects in males was 34 mg/kg/day (Lee et al. 1985; U.S. Army 1978b). In rodents, respective NOAEL and LOAEL values for reproductive effects (testicular atrophy and decrease or absent spermatogenesis) were 9–34 and 35–371 mg/kg/day in rats and 137–295 and 413–1,032 mg/kg/day in mice treated for 13 weeks (Bloch et al. 1988; Hong et al. 1985; Kozuka et al. 1979; Lee et al. 1985; U.S. Army 1978b, 1979).

Based on the available data, changes to hematological parameters in dogs exposed to oral 2,4-DNT for 9 months (Ellis et al. 1985; U.S. Army 1979) were evaluated as possible PODs for the intermediate-duration oral MRL for 2,4-DNT. To determine the POD, all available continuous-variable models in the EPA BMD Software (BMDS, version 2.1) were fit to the data for increased methemoglobin and reticulocytes and decreased hemoglobin, erythrocyte count, and hematocrit (see Appendix A for detailed description of BMD modeling). The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls. Neither the constant nor the non-constant variance model provided an adequate fit to the data for decreased erythrocytes or increased methemoglobin; therefore, these data were not considered suitable for BMD modeling. Models provided an adequate fit to data for decreased hematocrit and hemoglobin and increased reticulocytes. Among all of the models providing adequate fit to the data for these parameters, the lowest BMDL_{1SD} values were 0.67 mg/kg/day for hematocrit (exponential model 4), 3.66 mg/kg/day for hemoglobin (the exponential model 2), and 5.64 mg/kg/day for reticulocytes (polynomial 3-degree model); of these, the lowest BMDL_{1SD} of 0.67 mg 2,4-DNT/kg/day for decreased hematocrit was selected as the POD. This value was divided by an uncertainty factor of 100 (10 for animals to human extrapolation and 10 for human variability) resulting in an intermediate-duration oral MRL of 0.007 mg/kg/day.

Chronic-Duration Oral MRL for 2,4-DNT

- An MRL of 0.001 mg/kg/day has been derived for chronic-duration oral exposure (≥ 1 year) to 2,4-DNT.

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Information on effects of chronic-duration oral exposure is available from studies in dogs, rats, and mice. Results of these studies indicate that hematological, neurological, hepatic, and reproductive effects are associated with chronic-duration exposure to 2,4-DNT, with hematological effects and neurotoxicity as the most sensitive effects. Adverse hematological effects were observed in studies in dogs, rats, and mice, with the lowest NOAEL and LOAEL values of 0.2 and 1.5 mg/kg/day, respectively, observed following chronic oral exposure of dogs to 2,4-DNT (Ellis et al. 1985; U.S. Army 1979). Beagle dogs treated with 2,4-DNT at 1.5 and 10 mg/kg/day after 12 months developed hematological effects consistent with methemoglobinemia, anemia, and compensatory hematopoiesis (data are presented in Table A-3 of Appendix A). Female dogs administered 2,4-DNT at 1.5 mg/kg/day for 12 months showed statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin concentration. At 10 mg/kg/day, more pronounced changes in these hematological parameters were observed, including statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and a statistically significant increase in reticulocyte count. Similar hematological effects (decreased erythrocytes and decreased hematocrit) were observed in dogs treated with 0.2 mg/kg/day, but changes did not reach statistical significance. Although not statistically significant, effects on hematological parameters in male dogs were also similar to those seen in female dogs. In rodents, the lowest LOAEL for hematological effects was 3.9 mg/kg/day for decreased erythrocyte count (with no changes in methemoglobin or Heinz bodies) in male CD rats after treatment with 2,4-DNT for 12 months; the NOAEL for this effect was 0.6 mg/kg/day (Lee et al. 1985; U.S. Army 1978b, 1979).

Neurotoxicity was also observed in dogs, rats, and mice orally exposed to 2,4-DNT for chronic durations. In chronic-duration exposure studies, the lowest dose producing neurotoxicity was 1.5 mg/kg/day 2,4-DNT in a 2-year study in dogs; however, this effect (loss of hindquarter control) was only observed intermittently in one of six dogs. More severe signs of neurotoxicity and central nervous system lesions (vacuolization, hypertrophy, endothelial mitosis, focal gliosis in the cerebellum, and perivascular hemorrhage in the cerebellum and brain stem) occurred at 10 mg/kg/day (Ellis et al. 1985; U.S. Army 1979). In rodents, the lowest LOAEL values for neurotoxicity were 35 and 45 mg/kg/day for male and female CD rats, respectively, treated with 2,4-DNT for 1–2 years (Lee et al. 1985; U.S. Army 1978b, 1979). In mice, neurotoxicity was reported at 898 mg/kg/day (but not at 95 mg/kg/day) (Hong et al. 1985; U.S. Army 1979).

Hepatic effects have also been observed in laboratory animals following chronic-duration oral exposure to DNT. However, these effects are often observed in conjunction with the development of hepatocellular carcinoma and may represent precancerous changes. For example, dietary exposure of male rats to

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0.6 mg/kg/day 2,4-DNT induced “hepatocellular” alterations; however, this exposure level also induced neoplastic nodules (Lee et al. 1985). Thus, interpretation of data on hepatic toxicity is complicated due to the potential of 2,4-DNT to induce hepatocellular carcinoma. Biliary hyperplasia was observed in dogs exposed to 2,4-DNT for 24 months, with NOAEL and LOAEL values of 1.5 and 10 mg/kg/day, respectively (U.S. Army 1979). The lowest LOAEL value for hepatic effects in mice was 14 mg/kg/day for hepatocellular dysplasia following exposure to 2,4-DNT for 24 months (Hong et al. 1985; U.S. Army 1979). Hepatocellular degeneration, vacuolization, and altered hepatocellular foci were also observed in male F344 rats treated at 27 mg/kg/day for 52 weeks (Leonard et al. 1987).

Chronic-duration studies in laboratory animals have also demonstrated both male and female reproductive effects. The lowest LOAEL reported for reproductive effects is 14 mg/kg/day for atrophy of the testes and decreased spermatogenesis for in male CD-1 mice exposed to dietary 2,4-DNT for 12 months (Hong et al. 1985). Female mice fed 898 mg/kg/day 2,4-DNT had ovarian atrophy with non-functioning follicles, with no effects observed at 95 mg/kg/day (Hong et al. 1985). Male CD rats that received 34 mg/kg/day 2,4-DNT in the diet for 12 month showed an increased incidence of seminiferous tubule atrophy compared to controls (100% affected at the high-dose versus 0% of controls) (Lee et al. 1985; U.S. Army 1978b, 1979). No adverse reproductive effects were found in dogs fed 10 mg/kg/day 2,4-DNT for 24 months (Ellis et al. 1985; U.S. Army 1979).

Hematological effects and neurotoxicity were identified as the most sensitive effects of chronic-duration exposure to 2,4-DNT, with NOAEL and LOAEL values of 0.2 and 1.5 mg/kg/day, respectively, in dogs exposed to 2,4-DNT for 12 months (Ellis et al. 1985; U.S. Army 1979). For derivation of the chronic-duration oral MRL, hematological effects were selected as the critical effect rather than neurotoxicity, which was observed only intermittently in one of six dogs exposed to 1.5 mg/kg/day. Hematological data are expressed as group means; therefore, these data are considered more robust than observations of intermittent neurotoxicity in a single animal. To determine the POD, all available continuous-variable models in the EPA BMDS (version 2.1) were fit to the data for decreased erythrocyte count, hematocrit, and hemoglobin in female dogs. Hematological data from male dogs were not considered for additional BMD analyses due to the low number of dogs evaluated in the 10 mg/kg/day group (data were available for only two dogs). The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls. A detailed description of BMD modeling is provided in Appendix A. Neither the constant nor the non-constant variance model provided an adequate fit to the data for decreased hemoglobin; therefore, these data were not considered suitable for BMD modeling. Models provided an adequate fit to data for decreased erythrocyte count and

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decreased hematocrit. Among all of the models providing adequate fit to the data for these parameters, the lowest BMDL_{1SD} values were 0.12 mg/kg/day for hematocrit (exponential model 4) and 0.13 mg/kg/day for hemoglobin (exponential model 4); of these, the lowest BMDL_{1SD} of 0.12 mg/kg/day for decreased erythrocyte count was selected as the POD. This value was divided by an uncertainty factor of 100 (10 for animals to human extrapolation and 10 for human variability) resulting in a chronic-duration oral MRL of 0.001 mg/kg/day.

Acute-Duration Oral MRL for 2,6-DNT

- An MRL of 0.09 mg/kg/day has been derived for acute-duration oral exposure (≤ 14 days) to 2,6-DNT.

Information on effects of acute-duration oral exposure of laboratory animals to 2,6-DNT are available from a single-dose lethality studies in rats (Deng et al. 2011; Lee et al. 1975; U.S. Army 1978a; Vernot et al. 1977) and from an intermediate-duration study in dogs that evaluated clinical signs of toxicity, hematology, and clinical chemistry after 14 days of exposure (U.S. Army 1976). Results of these studies show that acute exposure to 2,6-DNT induces hematological effects, neurotoxicity, and hepatotoxicity, with hematological effects (methemoglobin-induced anemia and compensatory hematopoiesis) as the most sensitive effect. Results of acute lethality studies provide very little information on 2,6-DNT-induced toxicity at nonlethal levels.

Administration of 2,6-DNT to dogs at 20 and 100 mg/kg/day (but not 4 mg/kg/day) for 2 weeks produced hematological effects consistent with the development of methemoglobin-induced anemia and compensatory hematopoiesis (U.S. Army 1976). Dogs treated with 20 mg/kg/day showed a statistically significant decrease in erythrocyte count and a significant increase in mean cell hemoglobin. At 100 mg/kg/day, more pronounced changes in these hematological parameters were observed; dogs showed statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and a statistically significant increase in reticulocyte count. In the 4 mg/kg/day group, similar hematological effects (decreased erythrocyte count, hemoglobin, and hematocrit, and increased reticulocytes) were observed, but these changes did not achieve statistical significance. Data are presented in Table A-5 of Appendix A. No effects on clinical chemistry parameters were observed at any dose. Results of this study identify a NOAEL and a LOAEL of 4 and 20 mg/kg/day, respectively, for hematological effects in dogs treated with 2,6-DNT for 2 weeks. Although comprehensive end points were not evaluated after 2 weeks, the identification of hematological effects as the most sensitive effect after acute-duration

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exposure is consistent with the most sensitive effect identified after intermediate-duration exposure to 2,6-DNT.

In addition to hematological effects, clinical signs of neurotoxicity were observed in dogs administered 2,6-DNT at 100 mg/kg/day (U.S. Army 1976). The study authors noted that at least three dogs (gender not specified) developed neurotoxicity (listlessness, incoordination, lack of balance, and weakness, particularly of the hind limbs) and other clinical signs of toxicity (pale gums, dark urine) within the first 2 weeks of the exposure (incidence data were not reported). At this dose, one male dog died during the second week of exposure. No clinical signs of toxicity were observed at doses up to 20 mg/kg/day during the first 14 days of exposure, although similar (but milder) symptoms were reported at this dose following 4 weeks of treatment.

Hepatotoxicity, including increased alanine aminotransferase (ALT) activity and histopathological changes to the liver (congested sinusoids with sloughed hepatocytes and segmented neutrophils, disorganized midzonal regions characterized by infiltration of erythrocytes and hepatocytes with pyknotic nuclei and microvesiculated cytoplasm, and apoptotic hepatocytes) were reported in rats administered doses of 2,6-DNT (Deng et al. 2011). Results of this study identify acute-duration NOAEL and LOAEL values for hepatotoxicity for 2,6-DNT of 25 and 50 mg/kg/day, respectively.

Based on the available data, toxicity to the hematological system (anemia and compensatory hematopoiesis) was identified as the most sensitive effect of acute-duration oral exposure to 2,6-DNT. In dogs exposed for 14 days, a statistically significant decrease in erythrocyte count and a statistically significant increase in mean cell hemoglobin were observed after treatment with 2,6-DNT at 20 mg/kg/day for 2 weeks (U.S. Army 1976). Changes to other hematological parameters only reached statistical significance at 100 mg/kg/day. Therefore, the most sensitive hematological parameters were erythrocyte count and mean cell hemoglobin. To determine the POD for derivation of the acute-duration oral MRL for 2,6-DNT, all available continuous-variable models in the EPA BMDS (version 2.1) were fit to the data for erythrocyte count and mean cell hemoglobin. The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls. A detailed description of BMD modeling is provided in Appendix A. Neither the constant nor the non-constant variance model provided an adequate fit to the data for increased mean cell hemoglobin; therefore, these data were not considered suitable for BMD modeling. With the non-constant variance model applied, the linear, polynomial, and power models provided an adequate fit to the data for decreased erythrocyte count. The polynomial and power models converged to the linear model. The

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BMDL_{1SD} value of 9.31 mg/kg/day derived from this model was selected as the POD. This value was divided by an uncertainty factor of 100 (10 for animals to human extrapolation and 10 for human variability) resulting in an acute-duration oral MRL of 0.09 mg/kg/day.

Intermediate-Duration Oral MRL for 2,6-DNT

- An MRL of 0.004 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to 2,6-DNT.

Information on effects of intermediate-duration oral exposure of laboratory animals is available from a 13-week study in beagle dogs, CD rats, and CD-1 mice (U.S. Army 1976). Results of this study show that 2,6-DNT produces hematological, neurological, reproductive, and hepatic toxicity, with histopathological changes of the spleen (extramedullary erythropoiesis) that are likely secondary to methemoglobinemia and anemia.

Intermediate-duration oral exposure of beagle dogs to 2,6-DNT at 4 mg/kg/day (lowest tested dose) produced extramedullary erythropoiesis (formation of erythrocytes outside of the bone marrow) in the spleen secondary to methemoglobinemia and anemia (U.S. Army 1976). The incidence and severity of this lesion was dose-related. Data are presented in Table A-7 of Appendix A. Changes in hematological parameters associated with anemia and compensatory hematopoiesis, including decreased hematocrit and hemoglobin and increased numbers of reticulocytes, were observed at 20 and 100 mg/kg/day. However, mortality also occurred in dogs exposed to these doses. Results of this study identify a LOAEL of 4 mg/kg/day for extramedullary hematopoiesis secondary to hematological effects in dogs treated with 2,6-DNT for 13 weeks (U.S. Army 1976). In rodents exposed for 13 weeks, the lowest LOAELs for hematological effects (extramedullary erythropoiesis) were 7 mg/kg/day for rats and 51 mg/kg/day for mice (U.S. Army 1976).

Hepatic, neurological, and reproductive effects were also observed following a 13-week exposure of dogs, rats, and mice to 2,6-DNT (U.S. Army 1976); however, these effects occurred at doses higher than those producing extramedullary erythropoiesis (4 mg/kg/day) in dogs. Note that in dogs, lethality also occurred at doses of 20 and 100 mg/kg/day. The lowest NOAEL and LOAEL values for hepatic effects (bile duct hyperplasia and degeneration and inflammatory changes to the liver) were 4 and 20 mg/kg/day, respectively, in dogs treated with 2,6-DNT for 13 weeks (U.S. Army 1976). Bile duct hyperplasia was observed at higher doses in rats (35 mg/kg/day) and mice (51 mg/kg/day) fed 2,6-DNT for 13 weeks (U.S. Army 1976). Neurotoxic effects (listlessness, incoordination, and lack of balance) were observed in dogs

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treated with 20 and 100 mg/kg/day. No signs of neurotoxicity were observed in rats at doses up to 145 and 155 mg/kg/day (for males and females, respectively) or in mice at doses up to 289 and 299 mg/kg/day (for males or females, respectively) for 13 weeks (U.S. Army 1976). For reproductive effects following 13-week exposure to 2,6-DNT, the lowest NOAEL and LOAEL values of 4 and 20 mg/kg/day, respectively, were reported in dogs for testicular degeneration (U.S. Army 1976). NOAEL and LOAEL values for reproductive effects were 11 and 51 mg/kg/day, respectively, in male mice for decreased spermatogenesis and 7 and 35 mg/kg/day, respectively, in male mice for testicular atrophy (U.S. Army 1976).

The LOAEL value of 4 mg/kg/day for an increased incidence of extramedullary erythropoiesis in the spleens of dogs was identified as the POD for derivation of the intermediate-duration oral MRL for 2,6-DNT (U.S. Army 1976). Histopathology data were not suitable for BMD modeling, since the number of animals evaluated at each dose and time was small (n=2 animals). Therefore, the LOAEL value for 4 mg/kg/day was used at the POD. This value was divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability), resulting in an intermediate-duration oral MRL for 2,6-DNT of 0.004 mg/kg/day.

Chronic-Duration Oral MRL for 2,6-DNT

The only chronic exposure study identified for 2,6-DNT is a 1-year study in F344 rats (Leonard et al. 1987). In this study, oral exposure to 7 and 14 mg/kg/day 2,6-DNT produced hepatocellular carcinoma, cellular alterations to hepatocytes (vacuolization accompanied by acidophilic and basophilic foci), and increased liver weights and serum hepatic enzyme activity. Hepatocellular carcinoma was observed in 85 and 100% of rats treated with 7 and 14 mg/kg/day, respectively. No other effects of 2,6-DNT were reported in this study. Data from this study are not appropriate for derivation of a chronic-duration oral MRL for 2,6-DNT, as no noncancer effects were reported.

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3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

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

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

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

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the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

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

The primary focus of this document is information on 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT. However, some information on technical-grade DNT (Tg-DNT) is also provided. Tg-DNT contains approximately 76% 2,4-DNT, 19% 2,6-DNT, and <5% 3,4-, 2,3-, and 2,5-DNT. For each route of exposure (inhalation, oral, and dermal), human data will be discussed first. Data for each of the individual isomers (2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT, in that order) will be presented followed by data for Tg-DNT. Studies conducted in experimental animals will be discussed thereafter, with data on the individual isomers preceding data for Tg-DNT.

3.2.1 Inhalation Exposure

Most of the data on health effects associated with exposure of humans to DNT are from studies of workers exposed to 2,4-, 2,6-, or Tg-DNT. Exposure monitoring of workers in the past has generally been inadequate; consequently, few dose-response data based on human exposure to DNT isomers are available. No data were identified regarding inhalation exposure of humans to 2,3-, 2,5-, or 3,5-DNT. Regarding available animal data, one study was located that examined the acute inhalation toxicity of 2,6-DNT in experimental animals. No studies were located regarding inhalation exposure to 2,3-, 2,5-, or 3,5-DNT experimental animals.

Human exposure to chemicals in an occupational setting can occur via multiple routes: inhalation, dermal, and inadvertent ingestion (Hamill et al. 1982). Although the low vapor pressure of DNT makes inhalation of vapors unlikely, it can occur when contaminated particulate material is in the air. In

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addition, some dermal exposure is probable, and some ingestion may also occur as the result of eating or smoking without prior handwashing.

3.2.1.1 Death

In a retrospective cohort mortality study of 457 munitions workers who were exposed to either 2,4-DNT or Tg-DNT at two geographically different U.S. manufacturing plants, significant increases in death rates due to ischemic heart disease and residual diseases of the circulatory system were found (standardized mortality ratios [SMRs] of 126 and 143; 95% confidence intervals [CIs] of 65–234 and 112–179, respectively) (Levine et al. 1986a). Residual diseases of the circulatory system include congestive heart failure, cardiac arrest, and arteriosclerosis. The workers had been exposed to unreported concentrations of either 2,4-DNT (98% pure) or Tg-DNT for periods ranging from 30 days to >5 years (Levine et al. 1986a). Cigarette smoking was not taken into account in this study, but the study authors suggested that it may not have been a risk factor because mortality from lung cancer was less than expected. Among workers at both plants, there appeared to be a latency period of >15 years for a significant increase in mortality due to ischemic heart disease. There also appeared to be a relationship between heart disease and the intensity of exposure to DNT. No statistical increase was found in death due to cancer, either from malignant neoplasms as a whole or from individual cancers, although the statistical power of the study was insufficient to detect anything but gross changes in the death rate due to cancer.

The Levine et al. (1986a) retrospective cohort mortality study was limited by small cohort size, and thus, the study had diminished power to detect an effect. As a result, the finding of elevated mortality from heart disease among workers in two plants from different parts of the United States linked only by exposure to DNT is unusual. Workers in the United States generally have lower rates of heart disease than the general population because of the "healthy worker effect." At both plants, mortality from ischemic heart disease during the first 15 years following cohort entry was less than expected, and mortality increased only in later years. Suggestive, but not significant, is evidence of a relationship between heart disease and duration and intensity of exposure, also reported by Levine et al. (1986a). No studies were identified with respect to mortality after inhalation exposure of humans to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

In an acute-duration study, no mortality was observed in male or female F344 rats (5/sex/group) exposed nose-only to 2,6-DNT as a vapor at 26 mg/m³ for 6 hours and observed 14 days after dosing (CMA 1991). In the same study, groups of male and female rats exposed to 2,6-DNT at 0, 196, 473, or 694 mg/m³ as an

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aerosol exhibited mortality at ≥ 196 mg/m³. Two of five males (and no females) died at 196 mg/m³; all males and three of five females died at 694 mg/m³. Rats that died showed evidence of lung congestion and had increased relative lung weights compared to controls. An LC₅₀ of 0.43 mg/L was identified in rats, with LC₅₀ values for males and females of 0.24 and 0.66 mg/L, respectively.

No studies were located regarding death in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, or 3,5-DNT.

3.2.1.2 Systemic Effects

No studies were located regarding dermal, ocular, endocrine, or body weight effects in humans or animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT. No studies were located regarding respiratory effects in humans after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT or in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, or 3,5-DNT.

Respiratory Effects. No studies were identified with respect to respiratory effects after inhalation exposure of humans to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

In an acute-duration study, groups of male and female rats were exposed to 2,6-DNT at 0, 196, 473, or 694 mg/m³ as an aerosol for 6 hours and observed 14 days after dosing (CMA 1991). Rats exposed to 2,6-DNT as an aerosol at ≥ 196 mg/m³ exhibited signs of respiratory distress (exaggerated respiratory movements) for several days following exposure; recovery from this effect occurred by day 5 of the observation period. Rats that died showed evidence of lung congestion and had increased relative lung weights compared to controls.

No studies were identified with respect to respiratory effects after inhalation exposure of animals to 2,3-, 2,4-, 2,5-, or 3,5-DNT.

Cardiovascular Effects. No studies were identified with respect to cardiovascular effects after inhalation exposure of humans to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

Levine et al. (1986a) reported a significant increase in deaths from diseases of the circulatory system in workers involved in the manufacture and processing of 2,4-DNT and/or Tg-DNT. The preponderance of circulatory disease deaths was due to ischemic heart disease. The SMR was significantly elevated for

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ischemic heart disease when compared to the U.S. male population and to persons living in the area. When workers were divided by exposure duration, the significant increase in cardiovascular deaths was only observed in workers exposed for at least 5 months. Although the study did not control for cigarette smoking, a known risk factor for heart disease, the investigators noted that the study did not find significant increases in lung cancer or disease deaths.

No studies were located regarding cardiovascular effects in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Gastrointestinal Effects. Vomiting and nausea were mentioned as health complaints in a survey of male workers involved in the production of smokeless gunpowders during World War II (McGee et al. 1947). The exposure concentrations of 2,4-DNT were not specified. The investigators noted that prior to improving industrial hygiene practices, a much higher incidence of gastrointestinal symptoms was found. Since exposure to other compounds cannot be ruled out, attribution of these symptoms to DNT cannot be verified.

No studies were identified with respect to gastrointestinal effects after inhalation exposure of humans to 2,3-, 2,5-, 2,6-, or 3,5-DNT. No studies were located regarding gastrointestinal effects in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Hematological Effects. Several hematological effects, including anemia and cyanosis, were found in male workers employed by a munitions factory during World War II (McGee et al. 1947). In some cases, there were increases in leukocyte count, which may be related to prolonged exposure to DNT. The study authors presumed that the exposure concentrations to 2,4-DNT were relatively high because of the relatively primitive industrial hygiene practices at that time. Although 36 of 154 workers were anemic in the earlier study and 73 of 714 workers were anemic in the follow-up study, no control groups were used as a basis for comparison. Because of possible exposure to other compounds, lack of work histories, lack of exposure monitoring, lack of a control population, and small cohort size, the results obtained are equivocal and may be best used as qualitative descriptions of symptoms. Marked cyanosis and other incapacitating symptoms were reported after exposure to unspecified concentrations of Tg-DNT in a study of French workers in a DNT production plant during World War I (Perkins 1919). It is assumed that workers were exposed to high concentrations of Tg-DNT via both inhalation and dermal pathways, since the processes described involved direct handling of large amounts of Tg-DNT without protective equipment.

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No studies were identified with respect to hematological effects after inhalation exposure of humans to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

In an acute-duration study, male and female F344 rats (5/sex/group) were exposed to 2,6-DNT as a vapor at 0 or 26 mg/m³ or as an aerosol at 0, 196, 473, or 694 mg/m³ for 6 hours and observed 14 days after dosing (CMA 1991). Blood was analyzed for serum methemoglobin concentration 24 hours prior to dosing and 1, 24, and 48 hours and 7 days after dosing. Although the study authors noted slight increases in serum methemoglobin in 2,6-DNT-exposed animals in the first 24 hours after exposure, these changes were not clearly dose-related and were not statistically significantly different from control animals. No other hematological end points were evaluated.

No studies were located regarding hematological effects in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, or 3,5-DNT.

Musculoskeletal Effects. Joint pain, especially in the knees, and other incapacitating symptoms were found in unspecified numbers of French workers in a plant that produced DNT during World War I (Perkins 1919). No exposure concentrations were reported, but it is assumed that they were high because of the direct handling of large amounts of Tg-DNT without protective equipment, which also suggests that the workers were exposed dermally. However, because exposure to other compounds cannot be ruled out and no control data are available, caution must be used when interpreting these results.

No studies were located regarding musculoskeletal effects humans after inhalation exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT or in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Hepatic Effects. A study of 714 workers at a munitions plant found that 29 experienced liver tenderness (McGee et al. 1947). Other factors, such as alcohol consumption, may account for these results which should be viewed with caution because of the lack of control data, lack of information on exposure concentrations, and possible multiple chemical exposure. Medical surveys of 52 male workers exposed to Tg-DNT in a chemical plant that manufactured toluenediamine (TDA) revealed no differences in hepatic blood chemistry profiles (NIOSH 1982). Air samples contained concentrations ranging from 0.026 to 0.890 mg/m³ Tg-DNT (mean 0.207 mg/m³).

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No studies were located regarding hepatic effects in humans after inhalation exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT, or in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Renal Effects. No effects were observed on either of the renal parameters (blood urea nitrogen [BUN], creatinine) monitored in blood chemistry in a medical survey of 52 male workers exposed to Tg-DNT in a chemical plant that manufactured TDA (NIOSH 1982). Exposure concentrations in air samples taken for this study ranged from 0.026 to 0.890 mg/m³ Tg-DNT (mean 0.207 mg/m³). The study was limited by a small exposure population and lack of historical individual exposure monitoring.

Evidence of tubular and/or glomerular damage (alterations in urinary protein excretion patterns) was found among a cohort of approximately 160 workers at a copper mine exposed to Tg-DNT explosives (Brüning et al. 2001). Dividing the workers into exposure categories resulted in a significant dose-related trend for the incidence of tubular and/or glomerular damage among the workers without renal cell cancer or urothelial cancer. Approximately 80% of the 25 workers in the very high exposure category had evidence of renal damage. Dose-related increases in the excretion of α 1-microglobulins and glutathione-S-transferase- α were also observed suggesting proximal tubule damage. The lack of effect on glutathione-S-transferase- π levels suggested a lack of damage to the distal tubules.

No studies were located regarding renal effects in humans or animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans or animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

3.2.1.4 Neurological Effects

Dizziness and headache were reported by Perkins (1919) in a study of French workers exposed to Tg-DNT at a production plant during World War I. Although no exposure concentrations were reported, it is assumed that the workers were exposed to high concentrations of Tg-DNT via both inhalation and dermal pathways, since the manufacturing processes required workers to handle large amounts of Tg-DNT without protective equipment. Exposure to chemicals other than DNT in this environment could not be ruled out. Health effects of munitions workers exposed to unspecified levels of what was presumed to be 2,4-DNT were studied by McGee et al. (1947). Neurological signs reported by these

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workers included headache, dizziness, and pain, numbness, and tingling in the extremities. The 2,4-DNT exposure concentrations were not specified, but were considered by these authors to be relatively high as a result of the lack of safety practices.

No studies were located regarding neurological effects in humans after inhalation exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT or in animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

3.2.1.5 Reproductive Effects

Studies of men occupationally exposed to Tg-DNT at DNT and TDA plants showed no significant differences in sperm counts or morphology, follicle stimulating hormone (FSH) levels, or incidence of miscarriage in their wives compared to controls (Hamill et al. 1982; NIOSH 1982). In the NIOSH (1982) study, Tg-DNT concentrations ranged from 0.026 to 0.890 mg/m³ (mean 0.207 mg/m³). Interpretation of these studies is somewhat confounded by the lack of distinction between DNT and TDA exposure and the lack of information regarding exposure concentration in the Hamill et al. (1982) study. The limitations of these studies are similar (small exposure populations and the lack of individual exposure monitoring) and limit the ability of the studies to detect adverse effects.

No significant effects on the fertility of workers occupationally exposed to Tg-DNT have been found in several studies (Hamill et al. 1982; Levine et al. 1985a; NIOSH 1982). However, Levine et al. (1985a) estimate that only a 50–70% reduction in fertility could have been detected in the worker population that they studied.

One study by the CDC (1981) noted that sperm counts were decreased by >50% in workers in a Kentucky chemical plant exposed to DNT and TDA compared to workers unexposed to these chemicals. The study was limited because of multiple chemical exposures and the small numbers of workers examined. Thirty workers participated in the study: 9 currently exposed, 12 previously exposed, and 9 with no history of exposure to DNT/TDA.

No studies were located regarding reproductive effects in humans or animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

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3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

3.2.1.7 Cancer

The mortality of a cohort of 4,989 men who worked at least 5 months in a munitions facility was analyzed to determine whether DNT exposure was associated with an increased risk of cancer of the liver and biliary tract (Stayner et al. 1993). Workers were considered exposed if they had worked at least 1 day on a job with probable exposure to DNT. In this study, a significant increase in hepatobiliary cancer mortality (standard rate ratio [SRR]=3.88, 95% CI 1.04–14.41) was observed among DNT-exposed workers compared to unexposed control workers. However, no significant changes were noted when compared to the U.S. population, the SRR for hepatobiliary cancer being 2.67 (95% CI 0.98–5.83; $p=0.052$). No quantitative data were available on the DNT exposure of these men. This study is limited by the small numbers of hepatobiliary cancer cases, small numbers of workers with long exposure to DNT, and possible exposure of the workers to other chemicals. However, no significant increases in mortality from malignant neoplasms as a group or from particular cancers (liver, lung, gallbladder, kidney, and connective tissues) were observed in workers occupationally exposed to 2,4-DNT and/or Tg-DNT (Levine et al. 1986b). Exposures were not quantified and the cohort was small. The study authors estimated that an 8-fold increase in liver and gallbladder cancer in exposed workers would be necessary in order to be detected at the $p=0.05$ level; thus, the statistical analysis was not strong enough to detect small increases in cancer.

Among 500 workers in an underground copper mine where Tg-DNT-containing explosives were extensively used, increases, as compared to the German national cancer registry, in the incidence of renal cell cancer and urothelial cancer incidence were observed, (Brüning et al. 1999). However, when the workers were divided by exposure categories, no apparent relationship between renal cell cancer incidence and exposure category was found. In contrast, four of the six workers with urothelial cancer cases were in the high exposure group (one was in the lowest group and the other was in the highest exposure group). The genotype distribution of several polymorphic xenobiotic enzymes, including *N*-acetyltransferase 2 and glutathione-S-transferases M1 (GSTM1) and T1 (GSTT1), was examined in the 14 workers with renal cell cancer and 6 workers with urothelial cancer. The genotype distribution among the renal cell cancer cases did not differ from the normal German population. Similarly, the distribution of GSTM1 and GSTT1 genotypes for the urothelial cancer cases was similar to the German population.

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However, all of the urothelial cancer cases were found to be “slow acetylators” compared to a 58% distribution in the German population.

Harth et al. (2005) reported three cases of urothelial carcinoma of the urinary bladder among 60 workers exposed to Tg-DNT at a German explosive manufacturing facility. The investigators noted that this cancer rate was 15.9 times higher than the expected incidence in the federal state.

No studies were located regarding cancer in humans following inhalation exposure to 2,3-, 2,5-, or 3,5-DNT or in animals following inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

3.2.2 Oral Exposure

No studies were located regarding health effects in humans following oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT. However, it is assumed that oral ingestion could be a secondary route for occupationally exposed humans.

3.2.2.1 Death

No studies were located regarding death in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

2,4-DNT is lethal to experimental animals after oral administration. Animals generally developed cyanosis and ataxia after dosing. In general, rats are more sensitive than mice to the lethal effects of 2,4-DNT. The LD₅₀ values that have been determined for rats after gavage dosing with 2,4-DNT range from 270 to 650 mg/kg (U.S. Army 1975, 1978a; Vernot et al. 1977); in mice, LD₅₀ values were reported to be between 1,340 and 1,954 mg/kg after 2,4-DNT administration (U.S. Army 1975, 1978a; Vernot et al. 1977). In female Sprague-Dawley rats (5/group) administered a single dose of 2,4-DNT (in 5% v/v DMSO in corn oil) via gavage at 398 mg/kg and observed for 24 or 48 hours after dosing, two of five animals and one of five animals died within 24 and 48 hours, respectively (Deng et al. 2011). No deaths occurred in rats administered 2,4-DNT at 5, 50, 99, or 198 mg/kg. In a dominant lethal study by Lane et al. (1985), 8 of 15 male Sprague-Dawley rats died after receiving five daily doses of 240 mg/kg 2,4-DNT. No deaths were reported when male and female Sprague-Dawley rats were fed 78 or 82 mg/kg/day 2,4-DNT, respectively, in the diet for 14 days (McGown et al. 1983).

Death has been reported after intermediate- and chronic-duration exposure to 2,4-DNT in numerous studies. One of eight male and eight of eight female CD rats died after 3–13 weeks of ingesting 2,4-DNT

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in the diet (Lee et al. 1985; U.S. Army 1978b). Concentrations in the feed causing these deaths were equivalent to doses of 93 and 145 mg/kg/day in males and females, respectively. Death has also been reported in rodents fed concentrations equivalent to doses of 371–413 mg/kg 2,4-DNT in the diet for up to 6 months (Hong et al. 1985; Kozuka et al. 1979; U.S. Army 1978b). No treatment-related deaths were reported in rats fed up to 16.5 mg/kg/day 2,4-DNT or mice fed up to 28.5 mg/kg/day 2,4-DNT for 4 weeks, or in rats fed up to 22 mg/kg/day 2,4-DNT or mice fed up to 76 mg/kg/day 2,4-DNT for 78 weeks (NCI 1978). In a 3-generation reproductive study, there appeared to be an increased incidence of death among F₀ dams during parturition after receiving 45.3 mg/kg/day 2,4-DNT in the diet for 6 months (U.S. Army 1979). These deaths were associated with prolonged parturition, hemorrhage, and placental retention. However, because these effects were also seen to a lesser extent in control animals, it may be that the effects of 2,4-DNT simply enhanced effects caused by the advancing age of the dams (U.S. Army 1979).

In a 13-week study, some dogs fed 25 mg/kg/day became moribund after ≥ 22 days and had to be terminated, whereas no treatment-related deaths were reported in dogs fed 5 mg/kg/day (Ellis et al. 1985; U.S. Army 1978b). In addition to severe weight loss, severe neurological effects and histopathological changes were found in these animals, including vacuolization and focal gliosis in the cerebellum and perivascular hemorrhages in the cerebellum and brain stem, as well as peripheral neuropathy, testicular degeneration, and biliary hyperplasia. In a 24-month study of dogs, the administration of 10 mg/kg/day 2,4-DNT by capsule caused death within 6 months, but no deaths were reported at 1.5 mg/kg/day; clinical signs prior to death were similar to those reported in the 13-week study (Ellis et al. 1985; U.S. Army 1979). Decreased longevity was reported in 1–2-year studies of CD rats at average daily intakes as low as 3.9 mg/kg/day (males) and 5.1 mg/kg/day (females), and of CD-1 mice at 898 mg/kg/day (Hong et al. 1985; Lee et al. 1985; U.S. Army 1978b, 1979).

The experimental data are more limited for 2,6-DNT than for 2,4-DNT. After administration of 2,6-DNT, LD₅₀ values have been reported to range from 180 to 795 mg/kg in rats and from 621 to 807 mg/kg in mice (U.S. Army 1975, 1978a; Vernot et al. 1977). Complete lethality was reported in female Sprague-Dawley rats (5/group) administered a single dose of 2,6-DNT via gavage (in 5% v/v DMSO in corn oil) at 398 mg/kg (Deng et al. 2011). No deaths occurred in animals administered 5–199 mg/kg and observed for 2 days after dosing. The maximum tolerated dose (MTD) of 2,6-DNT corresponding to 100% survival of A/J mice after 6 doses over a 2-week period was 250 mg/kg (Schut et al. 1983).

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Intermediate-duration studies have also shown an increase in mortality of mice and dogs after 2,6-DNT administration. After feeding 51 mg/kg/day 2,6-DNT to male Swiss albino mice in the diet for up to 13 weeks, 8 of 16 of these animals died; 6 of 16 females fed 55 mg/kg/day 2,6-DNT also died (U.S. Army 1976). No treatment-related deaths were reported when rats were fed up to 155 mg/kg/day 2,6-DNT for the same duration (U.S. Army 1976). Two of eight dogs treated with 20 mg/kg 2,6-DNT by capsule died in a 13-week study (U.S. Army 1976). Thus, dogs seem to be the most sensitive of the three species to intermediate-duration oral 2,6-DNT exposure.

Vernot et al. (1977) reported LD₅₀ values for 2,3- and 2,5-DNT of 1,120 and 710 mg/kg/day, respectively, in male Sprague-Dawley rats and 1,070 and 1,230 mg/kg/day, respectively, in male CF-1 mice. No cause of death or additional information was reported.

Administration of up to 150 mg/kg/day Tg-DNT for 14 days was lethal to 6 of 13 pregnant F344 rats when administered by gavage during gestation (Jones-Price et al. 1982), yet this same concentration of Tg-DNT fed in the diet for 30 days did not kill any of the same strain of rats in another study (Hazleton Laboratories 1977). Decreased survival was found in CDF rats fed 35 mg/kg/day Tg-DNT for 52 weeks or 14 mg/kg/day Tg-DNT for 104 weeks (Hazleton Laboratories 1982).

No studies were located regarding the lethal effects of 3,5-DNT in experimental animals.

For 2,4-DNT, all LOAEL values from each reliable study for death in each species and duration category are recorded in [Table 3-1](#) and plotted in [Figure 3-1](#). For 2,6-DNT, all LOAEL values from each reliable study for death in each species and duration category are recorded in [Table 3-2](#) and plotted in [Figure 3-2](#).

3.2.2.2 Systemic Effects

The systemic effects observed after oral exposure of humans and animals to 2,4-DNT or 2,6-DNT are discussed below. No studies were located regarding systemic effects in humans or animals after oral exposure to 2,3-, 2,5-, or 3,5-DNT and no studies were located regarding musculoskeletal effects in humans or animals after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

For 2,4-DNT, the highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in [Table 3-1](#) and plotted in [Figure 3-1](#). For

Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
ACUTE EXPOSURE								
Death								
1	Rat (Sprague- Dawley)	5 d 1 x/d (GO)				240 M (8/15 died)	Lane et al. 1985 2,4-DNT	
2	Rat (CD)	once (GO)				568 M (LD50) 650 F (LD50)	U.S. Army 1975; U.S. Army 1978a 2,4-DNT	
3	Rat (Sprague- Dawley)	once (G)				270 M (LD50)	Vernot et al. 1977 2,4-DNT	
4	Mouse (Swiss albino)	once (GO)				1954 M (LD50) 1340 F (LD50)	U.S. Army 1975 2,4-DNT	
5	Mouse (CF-1)	once (G)				1630 M (LD50)	Vernot et al. 1977 2,4-DNT	
Systemic								
6	Rat (Sprague- Dawley)	once (GO)	Hepatic	50 F	99 F	Significantly decreased levels of serum albumin (13% lower than controls)	Deng et al. 2011 2,4-DNT	The NOAEL for renal effects is based on the absence of effects on levels of creatinine or urea in the serum.
			Renal	398 F				
			Bd Wt	198 F	398 F	Decreased body weight gain (1 to 4 g compared to 17 g for controls)		

Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	LOAEL				Reference Chemical Form	Comments
			System	NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)		
7	Rat (Sprague- Dawley)	5 d 1 x/d (GO)	Hemato		60 M (slight cyanosis)		Lane et al. 1985 2,4-DNT	
			Bd Wt	180 M	240 M (weight loss)			

Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
8	Rat (Sprague- Dawley)	14 d ad lib	Resp	260.9 M			McGown et al. 1983 2,4-DNT	
				272.7 F				
			Cardio	260.9 M				
				272.7 F				
			Gastro	260.9 M				
				272.7 F				
			Hepatic		78.3 M (increased alanine aminotransferase and cholesterol)			
					81.8 F (increased cholesterol)			
			Renal		78.3 M (hyaline droplet formation)			
					81.8 F (hyaline droplet formation)			
			Dermal	260.9 M				
				272.7 F				
			Ocular	260.9 M				
				272.7 F				

Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
Immuno/ Lymphoret								
9	Rat (Sprague- Dawley)	14 d ad lib		260.9 M 272.7 F			McGown et al. 1983 2,4-DNT	
Neurological								
10	Dog (Beagle)	12 d 1 x/d (C)		^b 5		25 (incoordination, stiffness, abnormal gait)	Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	
Reproductive								
11	Rat (Sprague- Dawley)	5 d 1 x/d (GO)		60 M		180 M (decreased fertility)	Lane et al. 1985 2,4-DNT	
12	Rat (Sprague- Dawley)	14 d ad lib				78.3 M (decreased thickness of spermatogenic sperm layers)	McGown et al. 1983 2,4-DNT	
13	Mouse DBA/2J	2 d 1 x/d (G)				250 M (decreased fertility)	Soares and Lock 1980 2,4-DNT	
INTERMEDIATE EXPOSURE								
Death								
14	Rat (Wistar)	6 mo ad lib (F)				371 M (71% died)	Kozuka et al. 1979 2,4-DNT	
15	Rat (CD)	4 or 13 wk ad lib (F)				93 M (1/8 died) 145 F (8/8 died)	Lee et al. 1985 2,4-DNT	

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Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
16	Rat (CD)	3 or 6 mo ad lib (F)				45.3 F (increased incidence of death during parturition)	U.S. Army 1979 2,4-DNT	
17	Mouse (CD-1)	4 or 13 wk ad lib (F)				413 (2/16M, 2/16F died))	Hong et al. 1985; U.S. Army 1978b 2,4-DNT	
18	Dog (Beagle)	4 or 13 wk 1 x/d (C)				25 (5/8 died)	Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	
19	Dog (Beagle)	6 mo 1 x/d (C)				10 M (4/6 died)	U.S. Army 1979, Ellis et al. 1985 2,4-DNT	
Systemic 20	Rat (Sprague- Dawley)	3 wk ad lib (F)	Bd Wt	76.7 M	156.4 M (10% decrease body weight)		Bloch et al. 1988 2,4-DNT	

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Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
21	Rat (Wistar)	6 mo ad lib (F)	Hemato		371 M (increased methemoglobin)		Kozuka et al. 1979 2,4-DNT	
			Hepatic		371 M (increased relative liver weight; increased SGOT, LDH, alkaline phosphatase, acid phosphatase, triglycerides, glucose; formation of puruloid matter)			
			Renal Bd Wt	371 M		371 M (41% decrease body weight)		
22	Rat (Fischer- 344)	6 or 26 wk ad lib (F)	Bd Wt		27 M (11% decrease body weight)		Leonard et al. 1987 2,4-DNT	

Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
23	Rat (CD)	4 or 13 wk ad lib (F)	Hemato	34 M	93 M (reticulocytosis; hemosiderosis)	266 M (anemia)	U.S. Army 1978b, Lee et al. 1985 2,4-DNT	
				38 F		145 F (anemia)		
					108 F (reticulocytosis; hemosiderosis)			
			Hepatic	266 M				
				145 F				
			Renal	266 M				
				145 F				
			Bd Wt			34 M (75% decrease body weight gain with decreased food consumption)		
						38 F (94% decrease body weight gain with decreased food consumption)		
24	Rat (CD)	3 or 6 mo ad lib	Bd Wt			34.5 M (23-25% decrease in body weight)	U.S. Army 1979 2,4-DNT	
						45.3 F (10-23% decrease in body weight)		

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Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
25	Mouse (CD-1)	4 or 13 wk ad lib (F)	Hemato	137 M	413 M		Hong et al. 1985; U.S. Army 1978b 2,4-DNT	
				147 F	468 F (mild anemia, reticulocytosis)			
			Hepatic	47 M		137 M		
				147 F		468 F (mild hepatocellular dysplasia)		
			Renal	413 M				
				468 F				
			Bd Wt			413 M		
						468 F (body weight loss with decreased food consumption)		

Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
26	Dog (Beagle)	4 or 13 wk 1 x/d (C)	Hemato	5		25 (anemia, Heinz bodies)	Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	
			Hepatic	25				
			Renal	25				
27	Dog (Beagle)	9 mo 1 x/d (C)	Hemato	1.5 M	10 M (increased Heinz bodies and methemoglobin)		U.S. Army 1979, Ellis et al. 1985 2,4-DNT	
				1.5 F				
				0.2 F ^c	10 F (decreased erythrocyte count, hemoglobin, and hematocrit; increased reticulocytes and Heinz bodies)			
					1.5 F (increased methemoglobin)			
Immuno/ Lymphoret								
28	Rat (Wistar)	6 mo ad lib (F)			371 M (increased relative spleen weight)		Kozuka et al. 1979 2,4-DNT	
29	Dog (Beagle)	4 or 13 wk 1 x/d (C)		25			Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	

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Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
Neurological								
30	Rat (Wistar)	6 mo ad lib (F)				347 M (humpback incoordination)	Kozuka et al. 1979 2,4-DNT	
31	Rat (CD)	4 or 13 wk ad lib (F)		34 M 108 F		93 M 145 F (demyelination of cerebellum and brain stem)(widespread and stiff-legged gait)	U.S. Army 1978b, Lee et al. 1985 2,4-DNT	
32	Mouse (CD-1)	4 or 13 wk ad lib (F)		413 M 468 F			Hong et al. 1985; U.S. Army 1978b 2,4-DNT	
33	Dog (Beagle)	4 or 13 wk 1 x/d (C)		5		25 (incoordination, abnormal gait, paralysis)	Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	
Reproductive								
34	Rat (Sprague- Dawley)	3 wk ad lib (F)			76.7 M (multinucleated spermatids, mild irregularity of basal lamina, vacuolation and lipid accumulation in Sertoli cells)	153.4 M (extensive degeneration of spermatids and spermatocytes; ultrastructural changes in Sertoli cells; 63% decrease sperm count)	Bloch et al. 1988 2,4-DNT	
35	Rat (Wistar)	6 mo ad lib (F)				371 M (testicular atrophy)	Kozuka et al. 1979 2,4-DNT	

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Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
36	Rat (CD)	13 wk ad lib (F)		9.3 M		93 M (decreased fertility)	U.S. Army 1978b, Lee et al. 1985 2,4-DNT	
37	Rat (CD)	4 or 13 wk ad lib (F)		34 M		93 M (severe decrease in spermatogenesis)	U.S. Army 1978b, Lee et al. 1985 2,4-DNT	
38	Rat (CD)	3 or 6 mo ad lib (F)				34.5 M 45.3 F (decreased fertility; difficult parturition)	U.S. Army 1979 2,4-DNT	
39	Rat (CD)	3 or 6 mo ad lib (F)		34.5 M		45 M (decreased fertility; severe atrophy/degeneration of seminiferous tubules)	U.S. Army 1979 2,4-DNT	
40	Mouse (CD-1)	4 or 13 wk ad lib (F)		137 M 468 F		413 M (mild degeneration of seminiferous tubules)	Hong et al. 1985; U.S. Army 1978b 2,4-DNT	
41	Mouse (albino- Swiss)	4 wk (C)		295 M		1032 M (decreased fertility index)	U.S. Army 1978b 2,4-DNT	

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Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
42	Dog (Beagle)	4 or 13 wk 1 x/d (C)		5 M 25 F		25 M (testicular degeneration, decreased spermatogenesis)	Ellis et al. 1985; U.S. Army 1978b 2,4-DNT	
Developmental								
43	Rat (CD)	3 or 6 mo ad lib (F)		5.1 F		45.3 F (difficult parturition)	U.S. Army 1979 2,4-DNT	
CHRONIC EXPOSURE								
Death								
44	Rat (CD)	1-2 yr ad lib (F)				3.9 M 5.1 F (decreased survival)	U.S. Army 1978b; U.S. Army 1979; Lee et al. 1985 2,4-DNT	
45	Mouse (CD-1)	24 mo ad lib (F)				898 (decreased survival)	U.S. Army 1979; Hong et al. 1985 2,4-DNT	
Systemic								
46	Rat (Fischer- 344)	52 wk ad lib (F)	Hepatic			27 M (hepatocellular degeneration and vacuolation; basophilic and acidophilic foci of cellular alteration)	Leonard et al. 1987 2,4-DNT	
			Bd Wt			27 M (25% body weight decrease)		

Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
47	Rat (Fischer- 344)	78 wk ad lib (F)	Bd Wt	8 M		20 M	NCI 1978 2,4-DNT	
				8.8 F		22 F (25% decrease body weight)(decrease body weight)		
48	Rat (CD)	1-2 yr ad lib (F)	Hemato	0.6 M	3.9 M (decreased RBC count)	34.5 M (anemia)	U.S. Army 1978b, 1979; Lee et al. 1985 2,4-DNT	
				5.1 F		45.3 F (anemia)		
			Renal	34.5 M				
				45.3 F				
			Bd Wt	3.9 M		34.5 M (30% decrease body weight with decreased food consumption)		
				5.1 F		45.3 F (27% decrease body weight with decreased food consumption)		
49	Mouse (C57BL/6N)	78 wk ad lib (F)	Bd Wt	14.4 M	72 M (18% decrease in body weight gain)	76 F (24% decrease in body weight gain)	NCI 1978 2,4-DNT	
					15.2 F (11% decrease in body weight gain)			

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Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
50	Mouse (CD-1)	24 mo ad lib (F)	Hemato	95		898 (anemia; reticulocytosis; Heinz bodies)	U.S. Army 1979; Hong et al. 1985 2,4-DNT	
			Hepatic	95 F		14 M (hepatocellular dysplasia) 898 F (hepatocellular dysplasia)		
			Renal			14 M (cystic dysplasia; toxic nephropathy)		
			Bd Wt	14 M 95 F	95 M (16% decrease in body weight)	898 F (20% decrease in body weight)		
51	Dog (Beagle)	24 mo 1 x/d (C)	Hemato	0.2	1.5 (methemoglobinemia, anemia)		U.S. Army 1979, Ellis et al. 1985 2,4-DNT	
			Hepatic	1.5	10 Biliary hyperplasia			
			Renal	10				
52	Dog (Beagle)	12 mo 1 x/d (C)	Hemato		0.2 ^d F (increased reticulocytes)		U.S. Army 1979, Ellis et al. 1985 2,4-DNT	

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Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
Neurological								
53	Rat (CD)	1-2 yr ad lib (F)				34.5 M (wide-spread and stiff-legged gait)	US Army 1978b, 1979; Lee et al. 1985 2,4-DNT	
						45.3 F (wide-spread and stiff-legged gait)		
54	Mouse (CD-1)	24 mo ad lib		95		898 (stiff-legged gait, hyperactivity)	U.S. Army 1979; Hong et al. 1985 2,4-DNT	
55	Dog (Beagle)	24 mo 1 x/d (C)		0.2		1.5 (loss of hindquarter control, convulsions)	U.S. Army 1979, Ellis et al. 1985 2,4-DNT	
Reproductive								
56	Rat (CD)	1-2 yr ad lib (F)		3.9 M	34.5 M Atrophy of seminiferous tubules, aspermato-genesis		U.S. Army 1978b, 1979; Lee et al. 1985 2,4-DNT	
57	Rat (CD)	1-2 yr ad lib (F)			34 M		U.S. Army 1978b; U.S. Army 1979; Lee et al. 1985 2,4-DNT	

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Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/Duration/Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
58	Mouse (CD-1)	24 mo ad lib		95 F		14 M (decreased spermatogenesis and degenerative change; testicular atrophy)	U.S. Army 1979; Hong et al. 1985 2,4-DNT	
						898 F (ovarian atrophy; nonfunctioning follicles)		
59	Dog (Beagle)	24 mo 1 x/d (C)		10 M			U.S. Army 1979, Ellis et al. 1985 2,4-DNT	
Cancer								
60	Rat (Fischer- 344)	78 wk ad lib (F)				7.5 M (CEL: skin and subcutaneous fibroma)	NCI 1978 2,4-DNT	
						22 F (CEL: mammary fibroadenoma)		
61	Rat (CD)	1-2 yr ad lib (F)				34.5 M (CEL: hepatocellular carcinoma; mammary and skin tumors)	U.S. Army 1978b, 1979; Lee et al. 1985 2,4-DNT	
						45.3 F (CEL: hepatocellular carcinoma, mammary and skin tumors)		

Table 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
62	Mouse (CD-1)	24 mo ad lib				95 M (CEL: renal solid carcinoma, cystic papillary carcinoma and adenoma, cystic adenoma)	U.S. Army 1979; Hong et al. 1985 2,4-DNT	

a The number corresponds to entries in Figure 3-1.

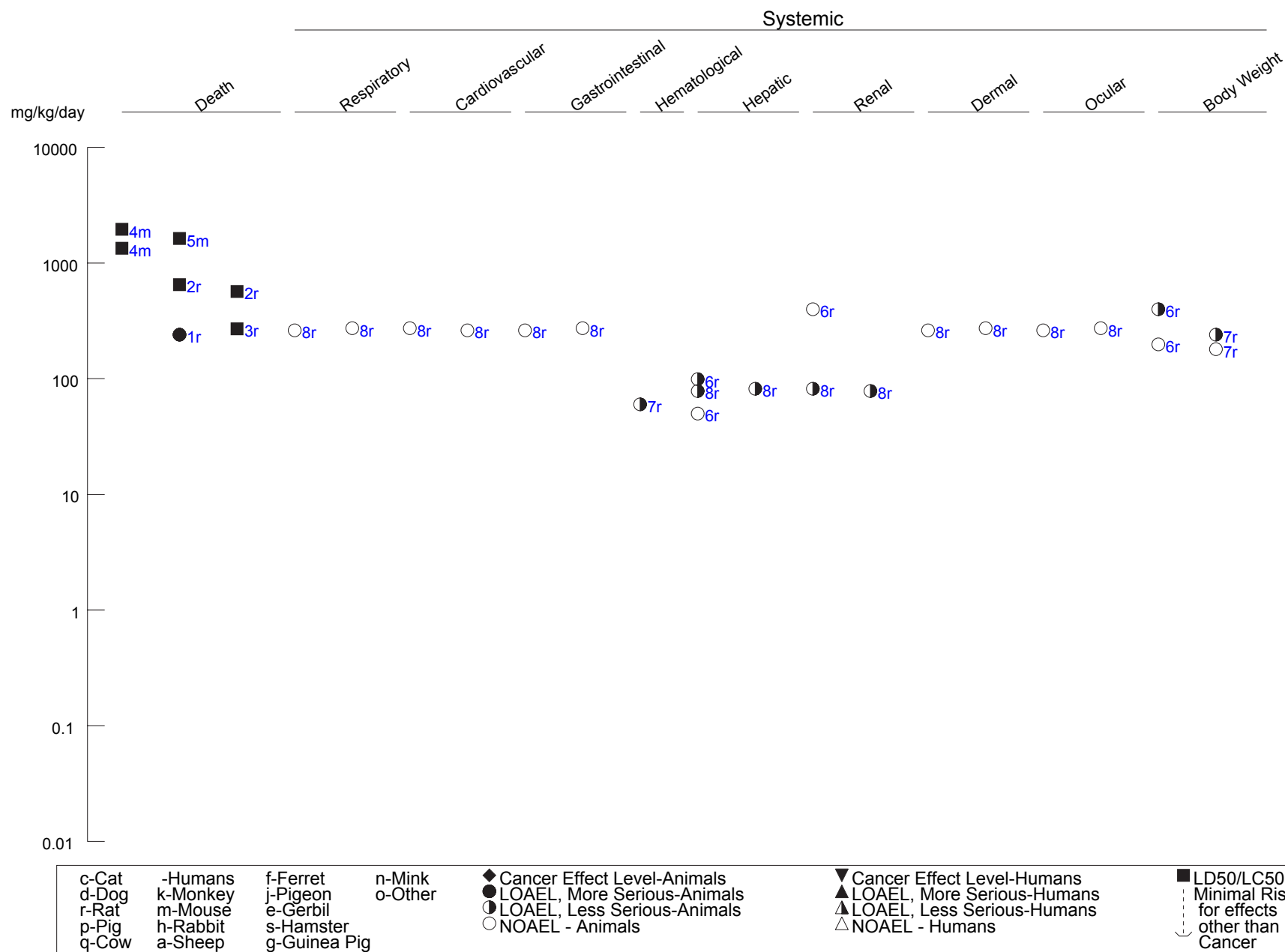
b Used to derive an acute-duration oral minimal risk level (MRL) of 0.05 mg/kg/day; the MRL was derived by dividing by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

c Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.007 mg/kg/day; the MRL was derived by dividing the BMDL1SD of 0.67 mg/kg/day by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

d Used to derive a chronic-duration oral minimal risk level (MRL) of 0.001 mg/kg/day; the MRL was derived by dividing the BMDL1SD of 0.12 mg/kg/day by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

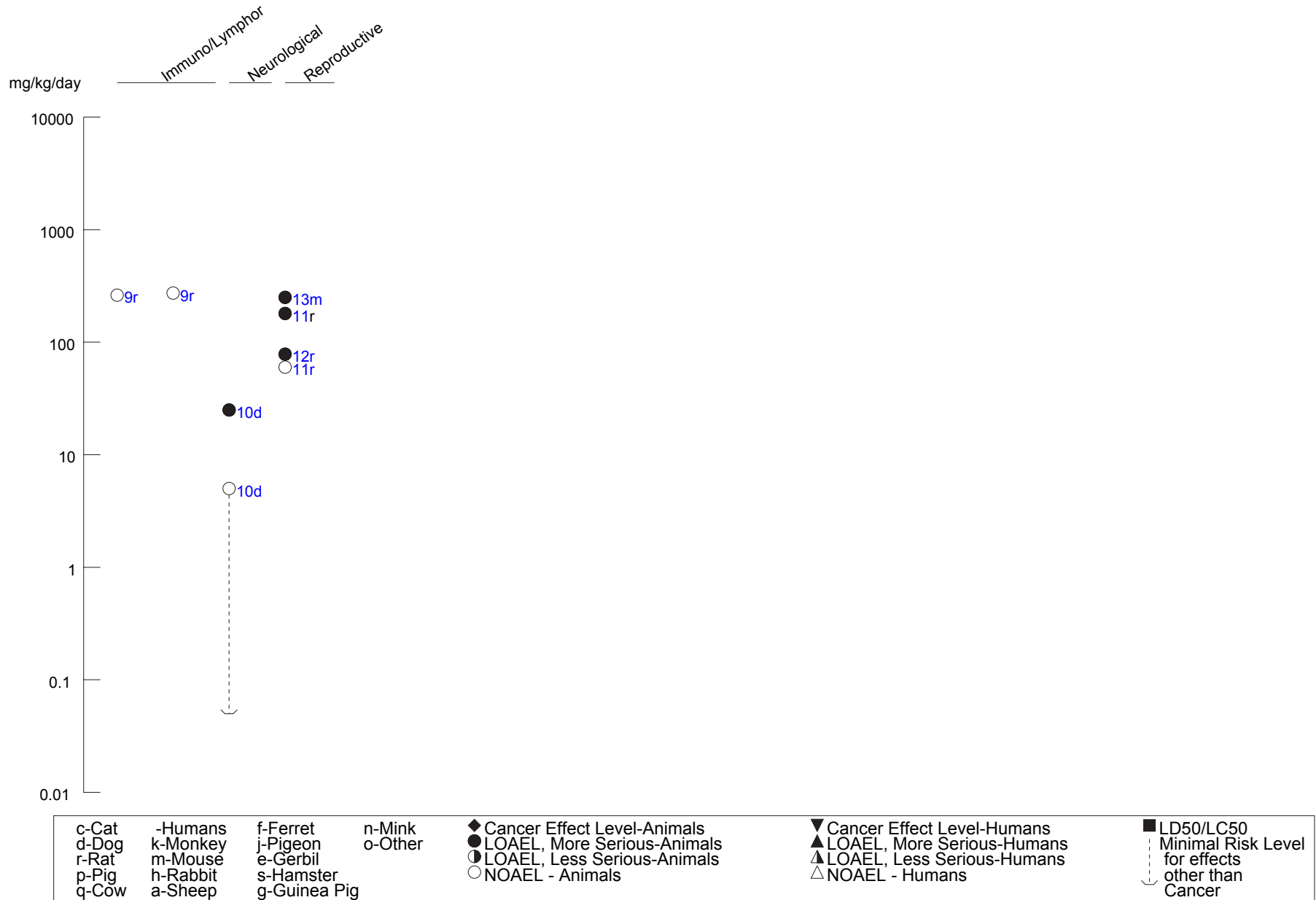
ad lib = ad libitum; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; (GO) = gavage in oil; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; RBC = red blood cell; Resp = respiratory; x = time(s); wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral
Acute (≤ 14 days)



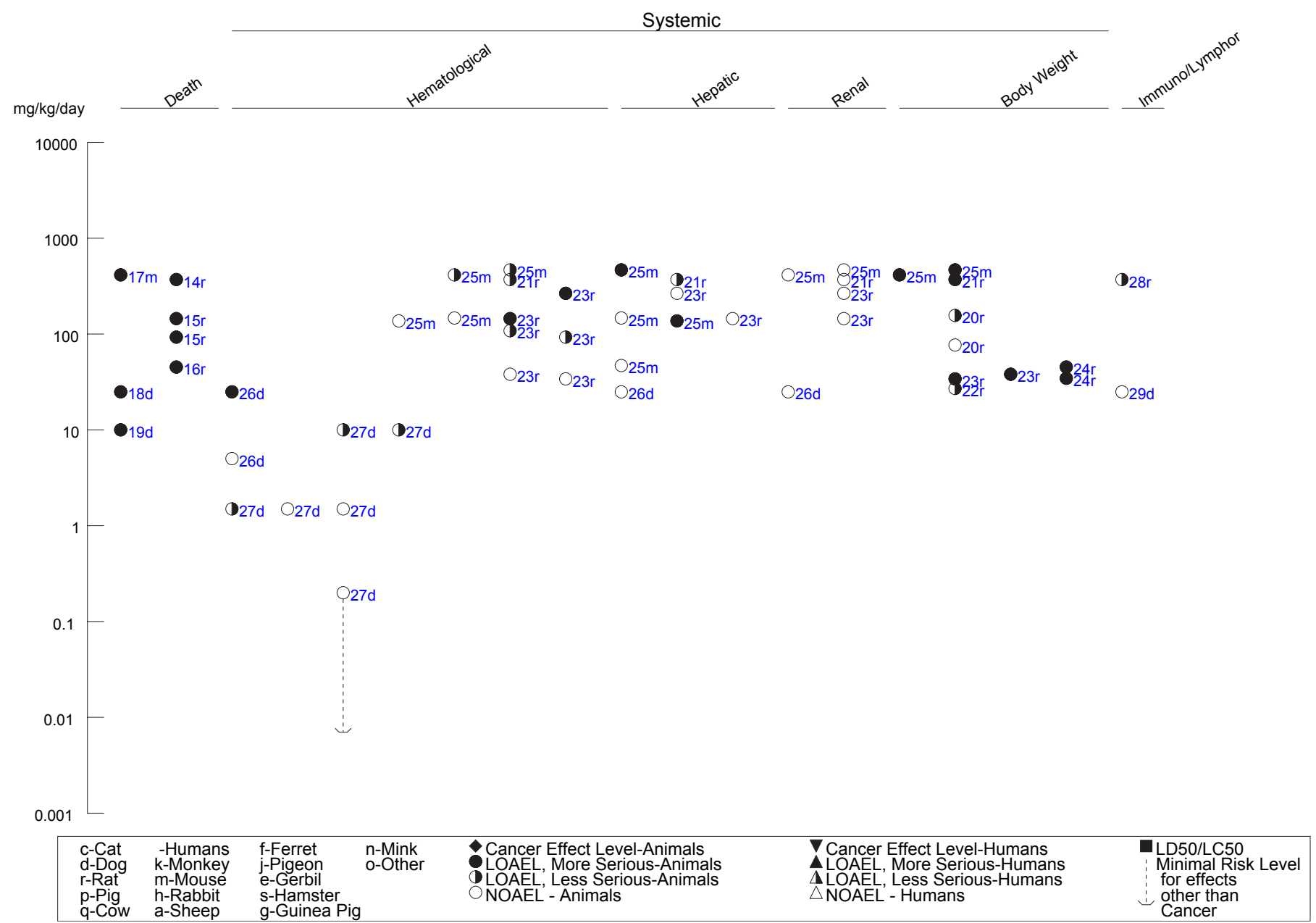
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Figure 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (*Continued*)
Acute (≤14 days)



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Figure 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (Continued)
Intermediate (15-364 days)



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Figure 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (Continued)
Intermediate (15-364 days)

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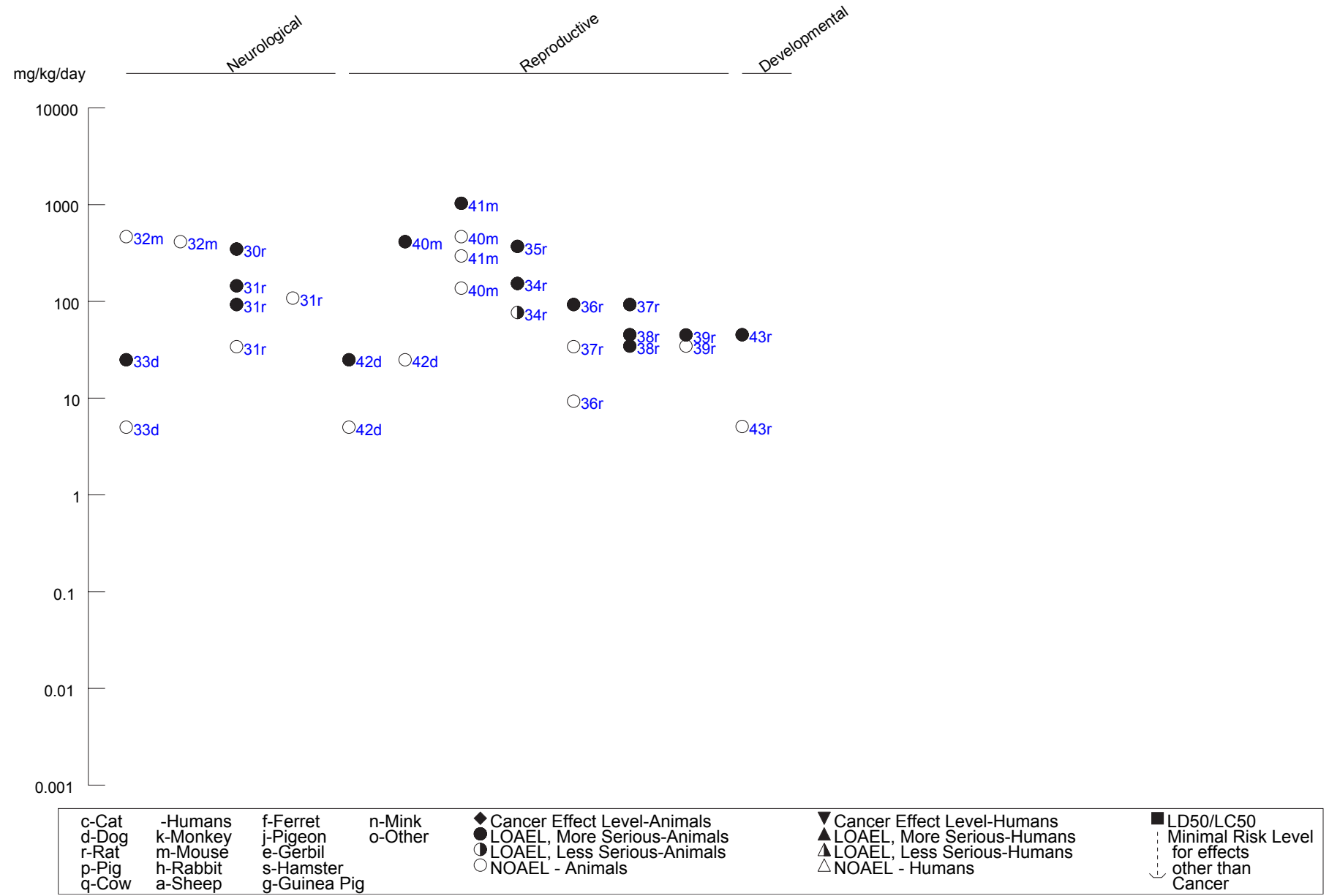
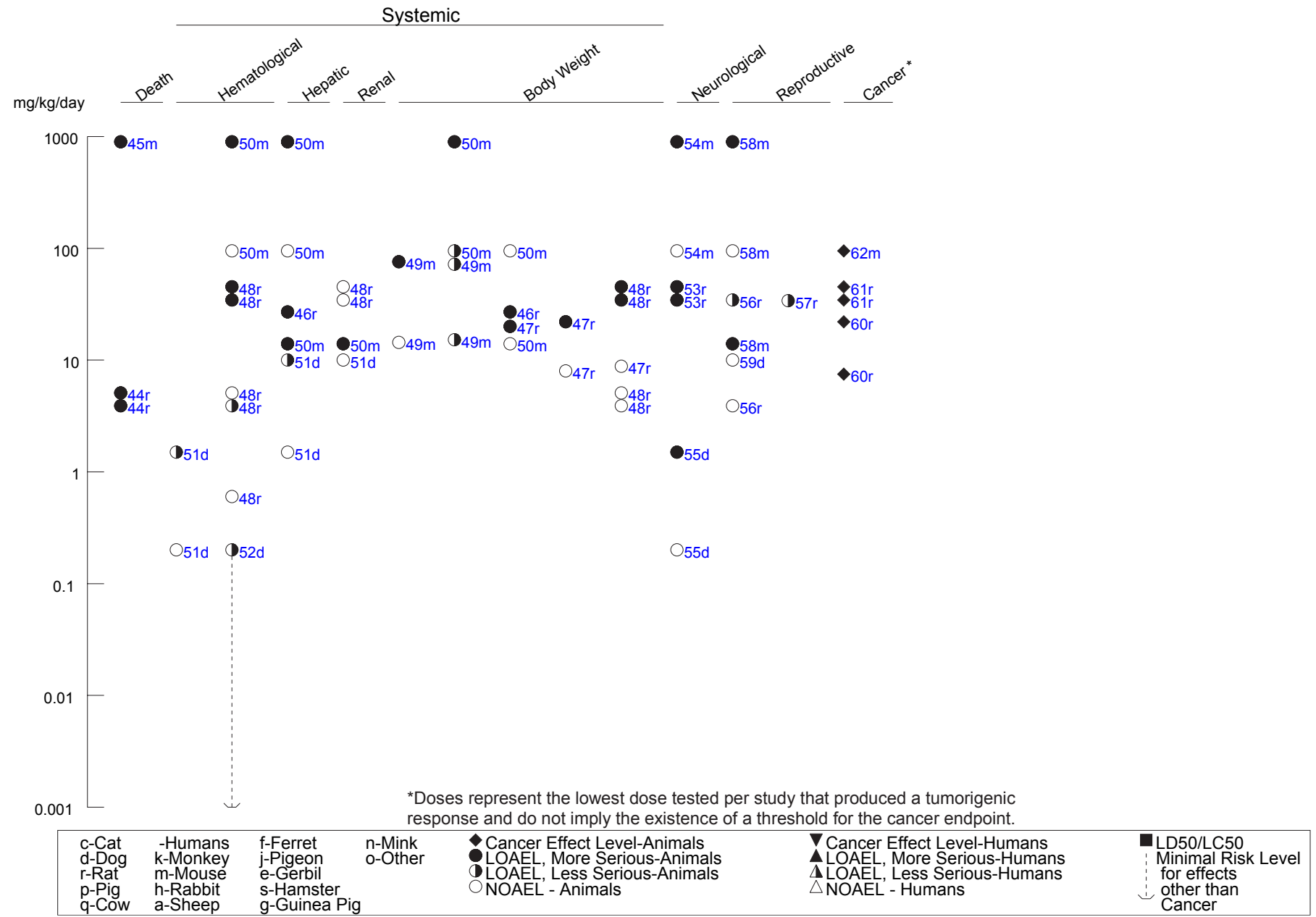


Figure 3-1 Levels of Significant Exposure to 2,4-Dinitrotoluene - Oral (Continued)
Chronic (≥365 days)



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Table 3-2 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
ACUTE EXPOSURE								
Death								
1	Rat (CD)	once (GO)				535 M (LD50) 795 F (LD50)	U.S. Army 1975; U.S. Army 1978a 2,6-DNT	
2	Rat (Sprague-Dawley)	once (G)				180 M (LD50)	Vernot et al. 1977 2,6-DNT	
3	Mouse (CD)	once (GO)				621 M (LD50) 807 F (LD50)	U.S. Army 1975 2,6-DNT	
4	Mouse (CF-1)	once (G)				1000 M (LD50)	Vernot et al. 1977 2,6-DNT	
Systemic								
5	Rat (Sprague-Dawley)	once (GO)	Renal	199 F			Deng et al. 2011 2,6-DNT	The NOAEL for renal effects is based on the absence of effects on levels of creatinine or urea in the serum.
			Bd Wt	25 F	50 F	Decreased body weight gain (1 to 4 g compared to 17 g for controls)		
6	Dog (Beagle)	2 wk ad lib (C)	Hemato	^b 4	20	(decreased erythrocytes and increased mean cell hemoglobin)	US Army 1976 2,6-DNT	

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Table 3-2 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
INTERMEDIATE EXPOSURE								
Death								
7	Mouse (Swiss- albino)	4 or 13 wk ad lib (F)				51 M (8/16 died)	US Army 1976 2,6-DNT	
						55 F (6/16 died)		
8	Dog (Beagle)	4 or 13 wk ad lib (C)				20 F (2/8 died)	US Army 1976 2,6-DNT	
Systemic								
9	Rat (Fischer- 344)	6 or 26 wk ad lib (F)	Bd Wt	7 M		14 M (20% decrease body weight)	Leonard et al. 1987 2,6-DNT	

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Table 3-2 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
10	Rat (CD)	4 or 13 wk ad lib (F)	Hemato	7 F	37 F (splenic hemosiderosis; extramedullary hematopoiesis)		U.S. Army 1976 2,6-DNT	
					7 F Increased incidence of extramedullary hematopoiesis of the spleen at 4 weeks			
					35 M (bile duct hyperplasia;hemosiderosi			
			Renal	145 M 155 F	37 F (bile duct hyperplasia; hemosiderosis)			
			Bd Wt	7	35 M	145 M		
					37 F (decreased body weight gain)	155 F (body weight loss)		

Table 3-2 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
11	Mouse (Swiss- albino)	4 or 13 wk ad lib (F)	Hemato	11 M	51 M (extramedullary hematopoiesis)		US Army 1976 2,6-DNT	
				11 F				
					55 F (extramedullary hematopoiesis)			
			Hepatic	11	51 M (bile duct hyperplasia)			
					55 F (bile duct hyperplasia)			
			Renal	289 M 299 F				
12	Dog (Beagle)	4 or 13 wk ad lib (C)	Bd Wt	11		51 M (weight loss)	US Army 1976 2,6-DNT	
						55 F (weight loss)		
			Hemato		^C 4 (mild extramedullary erythropoiesis)			
			Hepatic	4		20 (bile duct hyperplasia; degenerative and inflammatory liver changes)		
			Renal	4	20 (dilated tubules, degenerative foci)			
			Bd Wt	4		20 (body weight loss with decreased food consumption)		

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Table 3-2 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
Immuno/ Lymphoret								
13	Rat (CD)	4 or 13 wk ad lib (F)		145 M			U.S. Army 1976 2,6-DNT	
				155 F				
14	Dog (Beagle)	4 or 13 wk ad lib (C)		20	100 (thymic involution)		US Army 1976 2,6-DNT	
Neurological								
15	Rat (CD)	4 or 13 wk ad lib (F)		145 M			U.S. Army 1976 2,6-DNT	
				155 F				
16	Mouse (Swiss- albino)	4 or 13 wk ad lib (F)		289 M			US Army 1976 2,6-DNT	
				299 F				
17	Dog (Beagle)	4 or 13 wk ad lib (C)		4		20 (incoordination, lack of balance)	US Army 1976 2,6-DNT	
Reproductive								
18	Rat (CD)	4 or 13 wk ad lib (F)		7 M		35 M (decreased spermatogenesis; degeneration of testes)	U.S. Army 1976 2,6-DNT	
				155 F				

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Table 3-2 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral

(continued)

Key to Figure ^a	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
19	Mouse (Swiss- albino)	4 or 13 wk ad lib (F)		11 M 299 F		51 M (decreased spermatogenesis)	US Army 1976 2,6-DNT	
20	Dog (Beagle)	4 or 13 wk ad lib (C)		4 M 100 F		20 M (testicular degeneration)	US Army 1976 2,6-DNT	
CHRONIC EXPOSURE								
Systemic								
21	Rat (Fischer- 344)	52 wk ad lib (F)	Hepatic			7 M (hepatocellular degeneration, vacuolation; acidophilic and basophilic foci of cellular alteration)	Leonard et al. 1987 2,6-DNT	
			Bd Wt		7 M (18% decrease body weight)			
Cancer								
22	Rat (Fischer- 344)	52 wk ad lib (F)				7 M (CEL: cholangiocarcinoma, hepatocellular carcinoma)	Leonard et al. 1987 2,6-DNT	

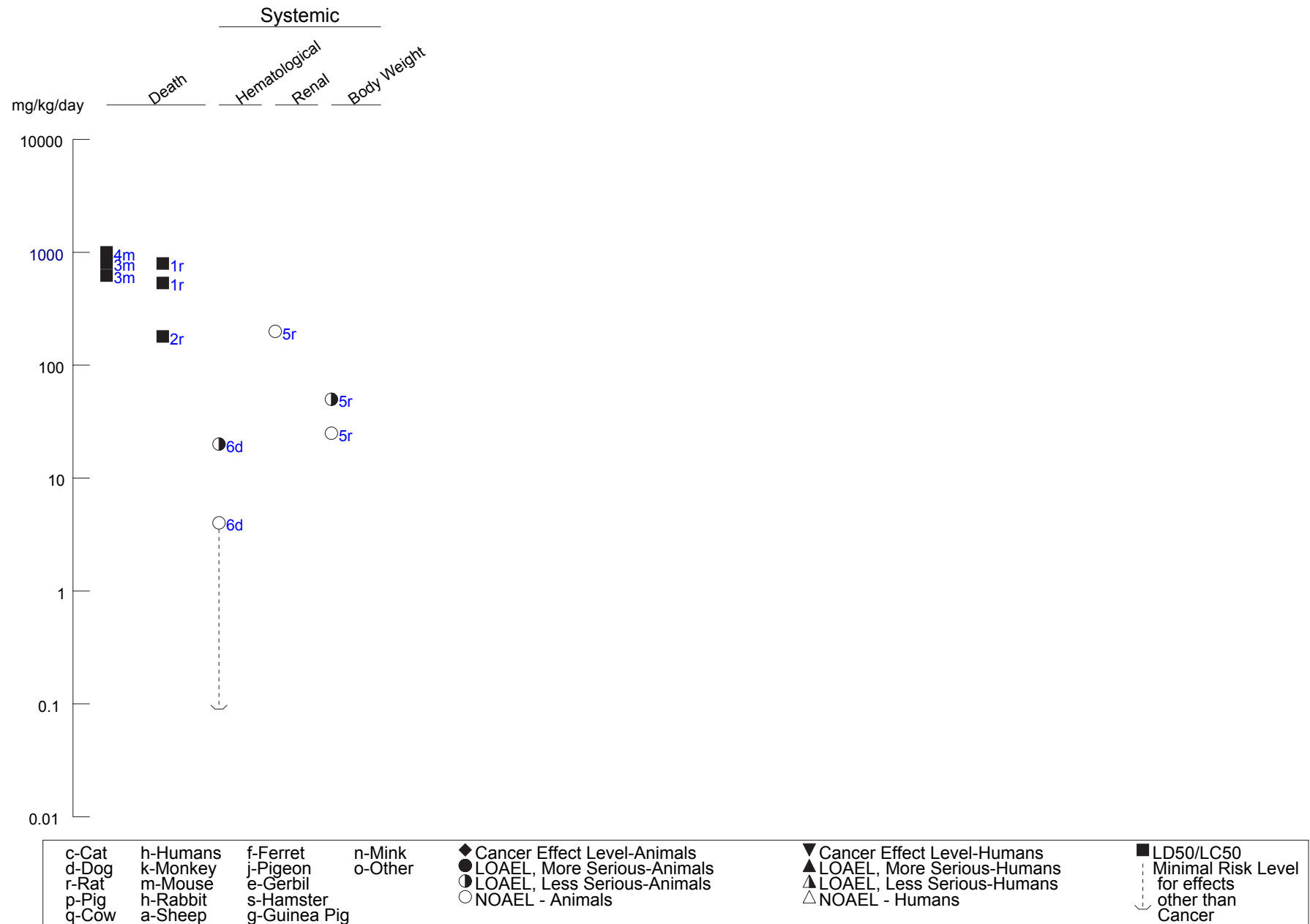
a The number corresponds to entries in Figure 3-2.

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.09 mg/kg/day; the MRL was derived by dividing the BMDL1SD of 9.31 mg/kg/day by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

c Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.004 mg/kg/day; the MRL was derived by dividing by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for animal-to-human extrapolation and 10 for human variability).

ad lib = ad libitum; Bd Wt = body weight; (C) = capsule; CEL = cancer effect level; (F) = feed; F = Female; (G) = gavage; (GO) = gavage in oil; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; wk = week(s)

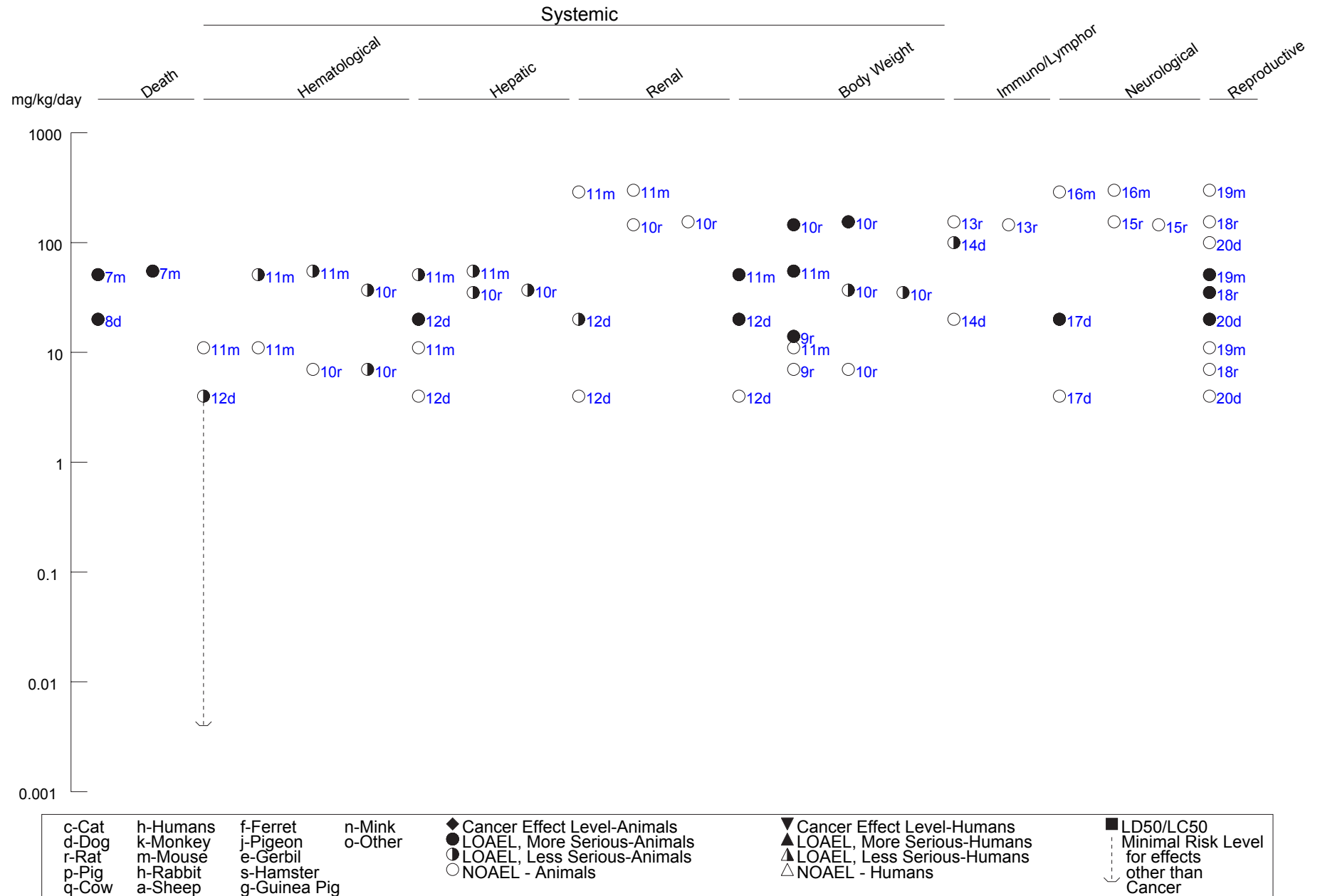
Figure 3-2 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral
Acute (≤14 days)



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Figure 3-2 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (*Continued*)

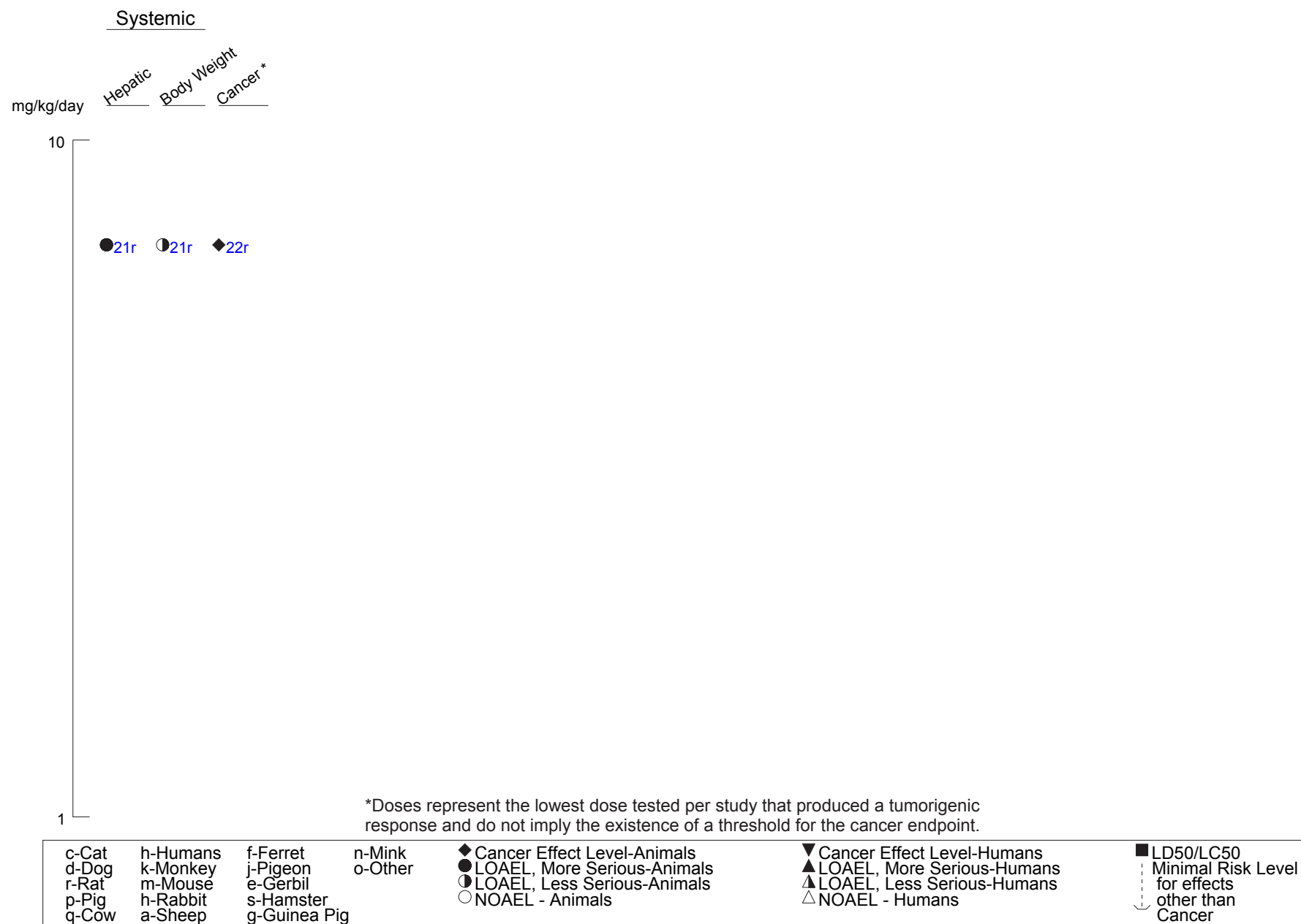
Intermediate (15-364 days)



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DINITROTOLUENES

3. HEALTH EFFECTS

Figure 3-2 Levels of Significant Exposure to 2,6-Dinitrotoluene - Oral (*Continued*)Chronic (≥ 365 days)

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3. HEALTH EFFECTS

2,6-DNT, all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in [Table 3-2](#) and plotted in [Figure 3-2](#).

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

No histopathological effects on the lungs were found when Sprague-Dawley rats were fed 261 mg/kg/day (males) or 273 mg/kg/day (females) 2,4-DNT for 14 days (McGown et al. 1983).

No respiratory system effects were observed when CDF rats were fed 14 mg/kg/day Tg-DNT for 2 years (Hazleton Laboratories 1982). Histopathological examination of the lungs and respiratory tract tissues of rats exposed to 14 mg/kg/day Tg-DNT for 2 years or 35 mg/kg/day for 1 year did not reveal any abnormalities (Hazleton Laboratories 1982).

No studies were located regarding respiratory effects in animals after oral exposures to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

No histopathological effects on the cardiovascular system were found after Sprague-Dawley rats received 261 mg/kg/day (males) or 273 mg/kg/day (females) 2,4-DNT in the diet for 14 days (McGown et al. 1983).

At the 26-week interim sacrifice in a 104-week study in which CDF rats were fed 0, 3.5, 14, or 35 mg/kg/day Tg-DNT in the diet, an increased incidence and severity of myocarditis was noted in males at 35 mg/kg/day (Hazleton Laboratories 1982). It was believed that this spontaneous inflammatory condition was exacerbated by ingestion of Tg-DNT in the high-dose animals. Although this condition was also observed at the 55-week sacrifice, it was not observed at 52 or 104 weeks.

No studies were located regarding cardiovascular effects in animals after oral exposures to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

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Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

There were no histopathological effects on the gastrointestinal tract of Sprague-Dawley rats fed 261 mg/kg/day (males) or 273 mg/kg/day (females) 2,4-DNT in the diet for 14 days (McGown et al. 1983).

Treatment of rats with up to 35 mg/kg/day Tg-DNT for up to 1 year or 14 mg/kg/day for up to 2 years did not cause any histopathological changes in the gastrointestinal tract (Hazleton Laboratories 1982).

No studies were located regarding gastrointestinal effects in animals after oral exposures to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Hematological effects were noted in virtually all animal studies of oral exposure to 2,4-DNT, 2,6-DNT, and Tg-DNT in which circulating blood was examined. The most common findings were methemoglobinemia, anemia, reticulocytosis, and an increase in Heinz bodies. The hematological effects are caused by oxidation of the iron in hemoglobin, producing methemoglobin. Heinz bodies are granules in erythrocytes that are believed to result from denatured hemoglobin. Reticulocytosis, a finding in many animals in these studies, is caused by the increased production of immature erythrocytes (red blood cells) and is seen as a compensatory mechanism in anemia resulting from exposure to 2,4- and 2,6-DNT. This hematotoxic syndrome is a common effect of exposure to aromatic amines and most organic and inorganic nitrates, and it has been implicated for many oxidizing agents (Smith 1996; U.S. Army 1979).

Female Sprague-Dawley rats (5/group) administered 2,4-DNT via gavage (in 5% v/v DMSO in corn oil) and observed for 24 or 48 hours after dosing showed evidence of erythrocytosis, as indicated by significant increases in hemoglobin, hematocrit, and/or erythrocyte and granulocyte counts, at doses ≥ 99 mg/kg (Deng et al. 2011). Relative to controls, erythrocyte and granulocyte counts were increased by 11 and 552% at 99 mg/kg after 24 hours; elevations in hemoglobin (27–31%) and hematocrit (29–33%) were only statistically significant in rats treated at 198 or 398 mg/kg and evaluated at 48 hours. No significant changes in hematological end points were observed in rats treated with 2,4-DNT at 5 or 50 mg/kg and evaluated 24 or 48 hours after dosing. Development of erythrocytosis 48 hours following a

3. HEALTH EFFECTS

single exposure to 2,4-DNT may be a secondary effect of dehydration, rather than a direct effect on the hematological system; however, no information on drinking water consumption was reported in this study. Slight cyanosis was observed in rats administered 60 mg/kg 2,4-DNT by gavage for 5 days (Lane et al. 1985). No changes in hematological parameters were found in Sprague-Dawley rats fed 261 mg/kg/day (males) or 273 mg/kg/day (females) 2,4-DNT in the diet for 14 days (McGown et al. 1983). Kozuka et al. (1979) found methemoglobin concentrations increased to 7 times those of controls in the blood of rats fed a time-weighted average (TWA) dose of 371 mg/kg/day 2,4-DNT in the diet for 6 months. Anemia was observed in a 13-week feeding study in which male and female CD rats were fed 266 and 145 mg/kg/day respectively in the diet; milder effects, such as reticulocytosis and hemosiderosis or abnormal pigment in the spleen, were found at 93 and 108 mg/kg/day in males and females, respectively (Lee et al. 1985; U.S. Army 1978b). No hematological effects were observed in males and females administered 34 and 38 mg/kg/day, respectively. Mild anemia (as indicated by decreases in erythrocyte count, hematocrit, or hemoglobin concentration) and concurrent reticulocytosis were also observed in male and female CD-1 mice administered 413 and 468 mg/kg/day 2,4-DNT, respectively, in the diet for 13 weeks (Hong et al. 1985; U.S. Army 1978b). Anemia, accompanied by the presence of Heinz bodies, was observed in beagle dogs given 25 mg/kg/day 2,4-DNT in capsules (Ellis et al. 1985; U.S. Army 1978b).

As part of a 2-year study in Beagle dogs (6/sex/group) exposed to 2,4-DNT at doses of 0.2, 1.5, or 10 mg/kg/day, effects on hematological parameters were evaluated after 3, 6, and 9 months of treatment (U.S. Army 1979). At these intermediate-duration timepoints, hematological effects consistent with development of methemoglobinemia, anemia, and compensatory hematopoiesis (including decreased hemoglobin, hematocrit, and erythrocyte counts, and increased serum methemoglobin and reticulocyte counts) were observed in beagle dogs administered oral 2,4-DNT at 1.5 or 10 mg/kg/day. In female dogs administered 10 mg/kg/day, statistically significant decreases in erythrocyte count, hematocrit, and hemoglobin, a statistically significant increase in reticulocyte count, and the presence of Heinz bodies in serum were observed. Similar hematological effects were observed in female dogs administered 0.2 and 1.5 mg/kg/day, although effects did not reach statistical significance, most likely because the power of the study to detect statistically significant changes was compromised by the small number of dogs per treatment group. However, a clinically significant increase in methemoglobin levels of 225% was observed in female dogs administered 1.5 mg/kg/day; no significant hematological effects were observed at 0.2 mg/kg/day. Although effects at all time points were qualitatively similar, hematological changes observed after 9 months of exposure were more consistent and pronounced than those observed at the 3- and 6-month time periods.

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Chronic studies of animals administered 2,4-DNT provide data that strengthen the weight-of-evidence supporting hematological effects. In a 24-month study, hematological effects consistent with development of methemoglobinemia, anemia, and compensatory hematopoiesis (including decreased hemoglobin, hematocrit, and erythrocyte counts, and increased serum methemoglobin and reticulocyte counts) were observed in beagle dogs (6/sex/group) administered oral 2,4-DNT at 1.5 or 10 mg/kg/day for 12 months of continuous dosing (U.S. Army 1979). No significant hematological effects were observed in dogs administered 2,4-DNT at 0.2 mg/kg/day. After treatment for 18 or 24 months, only slight or no anemia, near normal reticulocyte levels, no Heinz bodies, and minimal amounts of methemoglobin were detected, likely reflective of an adaptive response (Ellis et al. 1985; U.S. Army 1978b).

In a 2-year study (with a 1-year interim sacrifice) in which CD rats were fed 0.6, 3.9, or 34.5 mg/kg/day (males) or 0.7, 5.1, or 45.3 mg/kg/day (females) 2,4-DNT, significant decreases in red blood cell count were found in mid-dose males compared to controls, and anemia (as indicated by further reductions in red blood cell count, decreased hematocrit, decreased hemoglobin, and a compensatory increase in reticulocytes) was found in high-dose animals after 1 year (Lee et al. 1985; U.S. Army 1978b, 1979). No changes in methemoglobin or Heinz bodies were found. CD-1 mice that were administered 14, 95, or 898 mg/kg/day 2,4-DNT in the diet for 24 months were found to be anemic (as shown by significant reductions in erythrocytes and hemoglobin) at the high concentration, with compensatory increases in reticulocytes (Hong et al. 1985; U.S. Army 1979).

Female Sprague-Dawley rats (5/group) administered 199 mg/kg 2,6-DNT as a single dose via gavage (in 5% v/v DMSO in corn oil) showed evidence of erythrocytosis, as indicated by statistically significant increases in serum hemoglobin (45%), hematocrit (41%), and erythrocyte (61%) and granulocyte (11-fold) counts 48 hours after dosing (Deng et al. 2011). Increased numbers of reticulocytes (44% higher than controls), which were associated with mature erythrocytes containing Heinz bodies, were also observed in rats administered 199 mg/kg and evaluated at 24 hours. No significant changes in hematological end points were observed in rats administered 2,6-DNT at 5–99 mg/kg and evaluated 24 or 48 hours after dosing. Development of erythrocytosis 48 hours following a single exposure to 2,6-DNT may be a secondary effect of dehydration, rather than a direct effect on the hematological system; however, no information on drinking water consumption was reported in this study. As part of a 13-week study in Beagle dogs (4/sex/group) exposed to 2,6-DNT at doses of 4, 20, or 100 mg/kg/day, effects on hematological parameters were evaluated after 2 weeks of treatment (U.S. Army 1976). Dogs treated at 20 mg/kg/day showed a statistically significant decrease in erythrocyte count (16%) and a significant

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increase in mean cell hemoglobin (5%) after dosing for 2 weeks. At 100 mg/kg/day, more pronounced changes consistent with development of methemoglobinemia, anemia, and compensatory hematopoiesis were observed; dogs showed statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and an increase in reticulocyte count after 2 weeks of continuous dosing (U.S. Army 1976).

Subchronic administration (13 weeks) of 2,6-DNT in dogs and rats provide data that strengthen the weight-of-evidence supporting hematological effects. Significant hematological effects were observed in beagle dogs (4/sex/group) after administration of 2,6-DNT at 20 and 100 mg/kg/day, but not 4 mg/kg/day (U.S. Army 1976). Dogs treated at 20 mg/kg/day showed a statistically significant decrease in erythrocyte count (12%) after dosing for 4 weeks. At 100 mg/kg/day, more pronounced changes consistent with development of methemoglobinemia, anemia, and compensatory hematopoiesis were observed; dogs showed statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and an increase in reticulocyte count after 4 weeks of continuous dosing. Histopathological evaluation of the spleen showed an increased incidence of extramedullary erythropoiesis in dogs treated with 2,6-DNT at 4, 20, or 100 mg/kg/day for 4 or 13 weeks; this effect is an adaptive response to 2,6-DNT-induced methemoglobinemia and anemia.

Subchronic (13-week) administration of 2,6-DNT in CD rats induced changes in hematological parameters (measured at 4, 8, and 13 weeks) indicative of anemia and compensatory hematopoiesis (including significant decreases in erythrocytes, hematocrit, and hemoglobin and increased reticulocytes) at the highest tested dose (145 and 155 mg/kg/day for male and female rats, respectively) only; these effects were most pronounced after treatment for 4 weeks (U.S. Army 1976). No significant hematological changes were observed at 7 and 35 mg/kg/day (males) or 7 and 37 mg/kg/day (females). However, histopathological effects (extramedullary hematopoiesis and/or splenic hemosiderosis), indicative of an adaptive response to anemia and compensatory erythropoiesis, were observed in male and female rats administered 2,6-DNT at doses ≥ 7 mg/kg/day. Although histopathological effects (extramedullary hematopoiesis) were observed in CD-1 mice administered 2,6-DNT at ≥ 51 mg/kg/day (but not 11 mg/kg/day) for 4 or 13 weeks, no statistically significant changes in hematological parameters were seen at levels up to 289 mg/kg/day (males) or 299 mg/kg/day (females). The study authors indicated that some blood samples clotted, making hematological analyses impossible to perform. The small number of animals evaluated likely contributed to the identification of histopathological findings of the spleen in the apparent absence of 2,6-DNT-induced hematological effects. The 2,6-DNT isomer was not tested for hematological end points in studies of chronic duration.

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Hematological changes consistent with those observed in anemia were found in pregnant F344 rats administered 100 mg/kg Tg-DNT by gavage during gestation days 7–20 (Jones-Price et al. 1982). Administration of Tg-DNT to rats in the diet for 4 weeks (Hazleton Laboratories 1977) or 26 weeks (Hazleton Laboratories 1982) resulted in dose- and duration-related adverse effects on hematological parameters. In the 4-week study at 37.5 mg/kg/day, significant increases in reticulocytes and percentage of Heinz bodies were noted in both sexes and significant increases in methemoglobin levels were found in females; anemia was observed at 100 mg/kg/day in both sexes (Hazleton Laboratories 1977). Spleens of rats fed 150 mg/kg Tg-DNT for 30 days in the diet were altered in appearance; these alterations included discoloration, enlargement, and surface irregularity (Hazleton Laboratories 1977). An increased incidence of extramedullary hematopoiesis was noted in the splenic red pulp of male, but not female, rats fed 35 mg/kg/day Tg-DNT in the diet for 52 weeks (Hazleton Laboratories 1982). In rats sacrificed after 26 weeks in a 24-month study, no effects on hematological parameters were observed at 14 mg/kg/day Tg-DNT. However, at 35 mg/kg/day, there were increases in reticulocytes and methemoglobin and decreases in red blood cells along with hemosiderosis and extramedullary hematopoiesis in males, and increases in mean cell volume (MCV) in females (Hazleton Laboratories 1982). After 1 year, slight-to-moderate myeloid and erythroid hyperplasia was noted in the bone marrow of most male rats treated with 35 mg/kg/day Tg-DNT (Hazleton Laboratories 1982). In a 24-month study in which Tg-DNT was administered to rats in the diet, anemia was observed at 14 mg/kg/day in males but not in females; the NOAEL for this effect in males was 3.5 mg/kg/day Tg-DNT (Hazleton Laboratories 1982).

No studies were located regarding hematological effects in animals after oral exposure to 2,3-, 2,5-, or 3,5-DNT.

The consistent observation of adverse hematological effects following exposure of laboratory animals to DNT indicates that the blood is a primary target of DNT toxicity.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans or animals after oral exposure to 2,3-DNT, 2,4-DNT, 2,5-DNT, 2,6-DNT, 3,5-DNT, or Tg-DNT.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

The hepatotoxic effects of DNT have been consistently observed in animals. The liver appears to be a target organ of DNT toxicity, particularly when administered to rats, but hepatotoxic effects have also

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been observed in mice and dogs. Hepatic effects of DNT include liver discoloration and inflammation, alteration of hepatocytes, proliferation of bile duct epithelium, and hyperplastic foci. However, as discussed in Section 3.2.2.7 (Cancer), 2,4-, 2,6-, and Tg-DNT have been shown to induce hepatocellular carcinoma following chronic-duration oral exposure. Thus, hepatic effects observed at less-than-chronic exposure durations or at lower doses may represent early stages of progressive development to hepatic cancer.

In female Sprague-Dawley rats (5/group) administered 2,4-DNT via gavage (in 5% v/v DMSO in corn oil) and observed for 48 hours, significantly decreased levels of serum albumin (13–51% lower than controls) were observed at doses ≥ 99 mg/kg (Deng et al. 2011). Although relative liver weight was also significantly increased at 99 mg/kg, this effect was not observed at 198 or 398 mg/kg. Hepatic sinusoid congestion was observed in rats administered 398 mg/kg 2,4-DNT in the absence of other histopathological effects. Increased blood cholesterol was found in male and female Sprague-Dawley rats fed 78 or 82 mg/kg/day 2,4-DNT, respectively, in the diet for 14 days, and increased alanine aminotransferase levels were found in males (McGown et al. 1983). Blood glucose levels trended upward in all male and female groups in this study, but were increased significantly only in females fed 273 mg/kg/day.

Oral administration of 2,4-DNT for 13 weeks to rats (266 or 145 mg/kg/day in males and females, respectively) and dogs (25 mg/kg/day) did not result in liver toxicity (Ellis et al. 1985; U.S. Army 1978b). After 26 weeks of treatment, rats fed 27 mg/kg/day in the diet had significant increases in epoxide hydrolase (EH) activity, which is sometimes considered to be a phenotypic marker of neoplastic nodules; however, hepatocellular lesions did not develop in these animals when treatment was carried through 52 weeks (Leonard et al. 1987). Mild hepatocellular dysplasia was observed in mice fed 137 mg/kg/day (males) or 468 mg/kg/day (females) of 2,4-DNT for 13 weeks (Hong et al. 1985; U.S. Army 1978b).

Hepatic effects have also been observed in laboratory animals following chronic-duration oral exposure to DNT. However, these effects are often observed in conjunction with the development of hepatocellular carcinoma and may represent precancerous changes. For example, dietary exposure of male rats to 0.6 mg/kg/day 2,4-DNT induced “hepatocellular” alterations; however, this exposure to this and higher doses induced neoplastic nodules (Lee et al. 1985). Hepatocellular degeneration and vacuolation accompanied by acidophilic foci and occasional basophilic foci of cellular alteration were found in F344 rats fed 27 mg/kg/day 2,4-DNT for 52 weeks (Leonard et al. 1987). The incidences of focal areas of alteration were less in the 2,4-DNT-treated rats than they were in rats similarly treated with 2,6-DNT or

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Tg-DNT. Wistar rats fed a TWA dose of 371 mg/kg/day 2,4-DNT in the diet for 6 months had increased relative liver weights, formation of puruloid matter, and increased levels of serum glutamic-oxaloacetic transaminase (SGOT), lactate dehydrogenase (LDH), alkaline and acid phosphatase, triglycerides, and blood glucose levels compared to controls (Kozuka et al. 1979). In this study, the levels of serum albumin and the albumin/globulin ratios were decreased. Hepatocellular dysplasia was found in male and female CD-1 mice fed 14 or 898 mg/kg/day 2,4-DNT, respectively, for 24 months (Hong et al. 1985; U.S. Army 1979). Administration of 10 mg/kg/day 2,4-DNT for 24 months resulted in biliary hyperplasia in dogs; this effect was not seen in dogs administered 1.5 mg/kg/day (Ellis et al. 1985; U.S. Army 1979).

Increased ALT activity and histopathological changes to the liver were observed in individual female Sprague-Dawley rats administered 50 and 99 mg/kg 2,6-DNT (in 5% DMSO in corn oil) by gavage and observed for 48 hours (Deng et al. 2011). Serum ALT activity was significantly increased 7-fold compared to controls in rats administered 50 and 99 mg/kg 2,6-DNT, but no increase was observed at doses of 5–25 mg/kg. Histopathological changes to the liver observed in rats administered 2,6-DNT at 50 or 99 mg/kg included congested sinusoids with sloughed hepatocytes and segmented neutrophils, disorganized midzonal regions characterized by infiltration of erythrocytes and hepatocytes with pyknotic nuclei and microvesiculated cytoplasm, and apoptotic hepatocytes (severity of effects not specified). ALT levels were similar to controls and hepatocytes were mostly undamaged in rats administered 2,6-DNT at 199 mg/kg, although evidence of sinusoid congestion, occasional erythrocyte infiltration (with no signs of necrosis), and enlarged nuclei in several hepatocytes were noted. Six weeks of dietary consumption of 7 mg/kg/day 2,6-DNT caused a 380% increase in EH levels in rats but did not increase the level of DT-diaphorase (DTD) (Leonard et al. 1987). In the same study, both of these enzymes were elevated after 6 weeks of treatment with 14 mg/kg/day of 2,6-DNT. Dosing of rats, mice, and dogs with 2,6-DNT for 13 weeks resulted in liver toxicity (U.S. Army 1976). Bile duct hyperplasia was observed in rats fed 35 mg/kg/day and mice fed 51 mg/kg/day 2,6-DNT for 13 weeks (U.S. Army 1976). Liver degeneration and bile duct hyperplasia were observed in dogs dosed with 20 mg/kg/day 2,6-DNT but were not seen in dogs dosed with 4 mg/kg/day (U.S. Army 1976). After 52 weeks of treatment with 7 mg/kg/day 2,6-DNT, hepatocellular degeneration and vacuolation accompanied by acidophilic and basophilic foci of cellular alteration were found in F344 rats (Leonard et al. 1987).

Irregular liver surfaces were found in male F344 rats fed 37.5 mg/kg/day Tg-DNT for 30 days (Hazleton Laboratories 1977). Hepatocytic necrosis, nonsupportive pericholangitis, and periportal megalocytosis were found in CDF rats fed 14 mg/kg/day for 26 weeks, and when treatment of these animals was extended to 2 years, slight-to-severe biliary cirrhosis was found in males (Hazleton Laboratories 1982). It

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has been suggested that this latter lesion may be a precursor to cholangiocarcinoma (Hazleton Laboratories 1982). Hepatocytic degeneration and acidophilic and basophilic foci of cellular alteration were observed in F344 rats fed 35 mg/kg/day Tg-DNT in the diet for 52 weeks (Leonard et al. 1987). When administration of Tg-DNT was continued for 24 months, liver discoloration resulted at 3.5 mg/kg/day and liver nodules and malignancies at 14 mg/kg/day (Hazleton Laboratories 1982).

No studies were located regarding hepatic effects in animals after oral exposure to 2,3-, 2,5-, or 3,5-DNT.

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Dawley rats (5/group) administered 2,4-DNT (in 5% DMSO in corn oil) via gavage at doses up to 398 mg/kg and evaluated at 24 or 48 hours showed no effects on levels of serum creatinine or urea (Deng et al. 2011). Hyaline droplet accumulation in the epithelium of the proximal convoluted tubule was found in both sexes of Sprague-Dawley rats after they were administered 78, 104, 165, or 261 mg/kg/day 2,4-DNT (males) or 82, 109, 173, or 273 mg/kg/day 2,4-DNT (females) in the diet (McGown et al. 1983). Although this effect was observed at all concentrations, there was no dose response evident. Oral administration of 2,4-DNT to mice (413 mg/kg/day), rats (145 mg/kg/day), and dogs (25 mg/kg/day) for 13 weeks did not result in significant adverse effects in the kidney (Hong et al. 1985; U.S. Army 1978b). Treatment of the same species for 24 months resulted in renal dysplasia in male mice at a dose of 14 mg/kg/day of 2,4-DNT, but no renal effects were observed in rats or dogs dosed with 34.5 mg/kg/day or 10 mg/kg/day, respectively (U.S. Army 1979). Adverse effects in the kidneys of mice included cystic dysplasia in the tubular epithelium, atypical epithelium lining the cysts, and a variety of tumors (Hong et al. 1985). These effects were more pronounced in male mice than in female mice.

Female Sprague-Dawley rats (5/group) administered 2,6-DNT (in 5% DMSO in corn oil) via gavage at doses up to 199 mg/kg showed no effects on levels of serum creatinine or urea at 24 or 48 hours (Deng et al. 2011). Dosing of dogs with 20 mg/kg/day 2,6-DNT for 13 weeks resulted in dilated tubules, foci of inflammation, and degeneration of the kidney (U.S. Army 1976). No treatment-related effects on the kidney were found when rats were fed 2,6-DNT for 13 weeks (U.S. Army 1976). The severe renal effects observed after 2,4-DNT administration in mice were not observed when mice were fed 289 mg/kg/day 2,6-DNT for 13 weeks (U.S. Army 1976). However, the renal toxicity of 2,4-DNT in mice was observed only after chronic administration. Chronic studies of 2,6-DNT have not been performed in mice.

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After 26 or 52 weeks of dietary consumption of 35 mg/kg/day Tg-DNT, BUN levels were significantly increased in CDF rats (Hazleton Laboratories 1982). Exacerbation of chronic interstitial nephritis that was also observed in controls was observed at 14 mg/kg/day Tg-DNT in a chronic study in rats (Hazleton Laboratories 1982).

No studies were located regarding renal effects in animals after oral exposure to 2,3-, 2,5-, or 3,5-DNT.

The kidney does not appear to be a sensitive target of DNT toxicity for all species tested. Severe renal effects were observed only in CD-1 mice fed 14 mg/kg/day 2,4-DNT for 24 months, and less severe renal effects were observed in dogs administered 20 mg/kg/day 2,6-DNT for 13 weeks.

Endocrine Effects. No studies were located regarding endocrine effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Administration of 2,4-DNT in the diet for 14 days, at 78 mg/kg/day for males or 82 mg/kg/day for females did not cause any histopathological changes in adrenal, pituitary, or thyroid glands of Sprague-Dawley rats (McGown et al. 1983).

No histopathological effects on adrenal, pituitary, or thyroid glands were found in rats treated with 14 mg/kg/day Tg-DNT for up to 2 years or 35 mg/kg/day for 1 year (Hazleton Laboratories 1982). Increases in the incidence and severity of parathyroid hyperplasia (males) and increases in the incidence and severity of fatty metamorphosis and vascular ectasia (males and females) were found in rats fed 14 mg/kg/day Tg-DNT in the diet in a chronic study (Hazleton Laboratories 1982).

No studies were located regarding endocrine effects in animals after oral exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

Dermal Effects. No studies were located regarding dermal effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Concentrations of up to 261 mg/kg/day 2,4-DNT for males or 273 mg/kg/day 2,4-DNT for females administered in the diet for 14 days to Sprague-Dawley rats caused no histopathological changes in their skin (McGown et al. 1983).

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No effects were found on the skin of rats treated for up to 2 years with 14 mg/kg/day Tg-DNT or up to 1 year with 35 mg/kg/day Tg-DNT (Hazleton Laboratories 1982).

No studies were located regarding dermal effects in animals after oral exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

Ocular Effects. No studies were located regarding ocular effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

The eyes of male and female Sprague-Dawley rats administered up to 261 or 273 mg/kg/day 2,4-DNT, respectively, in the diet for 14 days did not exhibit any alterations upon histopathological examination (McGown et al. 1983).

No effects were found on the eyes of rats treated for up to 2 years with 14 mg/kg/day Tg-DNT in feed or up to 1 year with 35 mg/kg/day Tg-DNT in feed (Hazleton Laboratories 1982).

No studies were located regarding ocular effects in animals after oral exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

Body Weight Effects. No studies were located regarding body weight effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Adverse effects on body weight and body weight gain in rats, mice, and dogs were observed after oral administration of 2,4-DNT, 2,6-DNT, and Tg-DNT. In most of these studies, a concurrent decrease in food consumption was also observed. Because exposure resulted from intake of the test article in feed in most of these studies, it is possible that some of the body weight changes resulted from inpalatability.

Adverse effects on body weight, including body weight loss, have been reported after almost all acute-, intermediate-, and chronic-duration oral administration of 2,4-DNT (Bloch et al. 1988; Deng et al. 2011; Ellis et al. 1985; Hazleton Laboratories 1982; Hong et al. 1985; Kozuka et al. 1979; Lane et al. 1985; Lee et al. 1985; Leonard et al. 1987; McGown et al. 1983; NCI 1978; U.S. Army 1978b, 1979). Female Sprague-Dawley rats (5/group) administered 2,4-DNT (in 5% DMSO in corn oil) via gavage at 398 mg/kg showed decreased body weight gain (1–4 g compared to 17 g for controls) 48 hours after dosing (Deng et al. 2011). In another acute study, rats dosed by gavage with 240 mg/kg 2,4-DNT for 5 days lost

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weight (Lane et al. 1985). In general, there were losses of 10–40% in body weight in acute-, intermediate-, and chronic-duration studies in rats. After 6 months, decreases in body weight gain were noted in rats fed 27 mg/kg/day 2,4-DNT (Leonard et al. 1987), and a 25% decrease in body weight was seen in rats fed 34.5 mg/kg/day (Lee et al. 1985; U.S. Army 1978b, 1979). This reduction in body weight gain tended to become more pronounced when 2,4-DNT was continued for periods of 1–2 years (Leonard et al. 1987). Body weight was decreased 25% in rats that received 20 mg/kg/day 2,4-DNT in the diet for 78 weeks; the NOAEL in this study was 8 mg/kg/day (NCI 1978). Mice showed similar decreases in body weight after intermediate- and chronic-duration exposure, but the concentrations of the test article needed to evoke this effect were considerably higher than in rats (Hong et al. 1985; NCI 1978; U.S. Army 1978b). An 18–24% decrease in body weight was seen in rats receiving 72–76 mg/kg/day 2,4-DNT in the diet for 78 weeks (NCI 1978).

Female Sprague-Dawley rats (5/group) administered 2,6-DNT (in 5% DMSO in corn oil) via gavage at 50 or 99 mg/kg gained less weight than control animals over the 48-hour observation period (1–4 g compared to 17 g for controls); rats from the same study administered 2,6-DNT at 199 mg/kg lost weight over the course of 48 hours (Deng et al. 2011). Administration of 2,6-DNT also caused decreased body weight gain or body weight loss in rats, mice, and dogs at concentrations ranging from 14 to 145 mg/kg/day in intermediate-duration studies (U.S. Army 1976). Treatment with 7 mg/kg/day 2,6-DNT decreased body weight in rats at 52 weeks by 18% (Leonard et al. 1987).

A 29% decrease in absolute maternal weight gain was observed in dams fed 14 mg/kg/day Tg-DNT for 14 days during gestation (Jones-Price et al. 1982). Decreased body weight or decreased body weight gain was reported in rats at levels as low as 14 mg/kg/day Tg-DNT in intermediate- or chronic-duration studies (Hazleton Laboratories 1982). Other intermediate- and chronic-duration studies also confirmed these body weight effects (Hazleton Laboratories 1977; Leonard et al. 1987; NCI 1978).

No studies were located regarding body weight effects in animals after oral exposure to 2,3-, 2,5-, or 3,5-DNT.

Metabolic Effects. No studies were located regarding metabolic effects in humans after oral exposure to 2,4- or 2,6-DNT.

Female Sprague-Dawley rats (5/group) administered 2,4-DNT (at 198 or 398 mg/kg) or 2,6-DNT (at 199 mg/kg) via gavage (in 5% DMSO in corn oil) showed small but statistically significant reductions in

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sodium levels in the serum (4–11% lower than controls) 24 and/or 48 hours after dosing (Deng et al. 2011). Rats treated with 2,4-DNT at 398 mg/kg also had elevated levels of blood glucose (increased 2.6-fold) 48 hours after dosing.

No studies were located regarding metabolic effects in animals after oral exposure to 2,3-, 2,5-, or 3,5-DNT.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Testing for immunological effects of DNT is limited. No changes in serum concentrations of IgE were observed in rats and dogs administered 2,4-DNT at levels up to 206 and 25 mg/kg/day, respectively, for 13 weeks (Ellis et al. 1985; Lee et al. 1985; U.S. Army 1978b). In these studies, the rats received the test article in feed, while the dogs received it in capsules. No histopathological changes were found in the spleen or thymus of Sprague-Dawley male rats fed 78 mg/kg/day 2,4-DNT or female rats fed 82 mg/kg/day 2,4-DNT in the diet for 14 days (McGown et al. 1983).

Administration of 2,6-DNT to dogs (up to 100 mg/kg) and rats (up to 145 mg/kg/day) for 13 weeks resulted in no observable changes in IgE serum concentrations (U.S. Army 1976). IgE is the antibody associated with allergic or hypersensitive reactions, and so it may be expected that the human sensitizing potential of 2,4- and 2,6-DNT would be low. Involution of the thymus was noted when dogs were administered 100 mg/kg 2,6-DNT, but was not noted when they were administered 20 mg/kg 2,6-DNT by capsule for 13 weeks (U.S. Army 1976).

For 2,4-DNT, the highest NOAEL values and all LOAEL values from each reliable study for immunological/lymphoreticular effects in each species and duration category are recorded in [Table 3-1](#) and plotted in [Figure 3-1](#). For 2,6-DNT, the highest NOAEL values and all LOAEL values from each reliable study for immunological/lymphoreticular effects in each species and duration category are recorded in [Table 3-2](#) and plotted in [Figure 3-2](#).

No studies were located regarding immunological and lymphoreticular effects in animals after oral exposure to 2,3-, 2,5-, or 3,5-DNT.

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3.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Neurotoxicity appears to be a characteristic syndrome of DNT poisoning of animals. Neurotoxic symptoms, of decreased severity compared to dogs, were observed in mice and rats at doses higher than neurotoxic doses in dogs; however, the test article was administered in feed to rodents and in capsules to dogs.

In a subchronic study, beagle dogs (4/sex/group) administered 2,4-DNT at 1 or 5 mg/kg/day showed no signs of behavioral changes or clinical signs of neurotoxicity. Neurotoxicity was observed at 25 mg/kg/day, with signs of neurotoxicity (loss of hind leg control) first observed in a female dog on day 12 of treatment. Three additional male dogs showed similar signs (signs not specified) on day 14 of treatment. All dogs administered 25 mg/kg/day showed signs of neurotoxicity after treatment for 12–22 days. The onset and severity of toxic signs reportedly varied among dogs within the same treatment group; some dogs were moribund at the same time that others began experiencing symptoms. In individual dogs, symptom severity varied over time, with no duration-related pattern of severity. The specific neurotoxic effects in dogs affected within the first 14 days of treatment were not specified. The NOAEL for the neurotoxicity observed after 12 days was 5 mg/kg/day. No histopathological changes were found in the brain or spinal cord of male and female Sprague-Dawley rats fed 2,4-DNT for 14 days in the diet at doses of 78 and 82 mg/kg/day, respectively (McGown et al. 1983).

Neurotoxicity has been reported in laboratory animals after intermediate- or chronic-duration exposure to 2,4-DNT with symptoms ranging from tremors, convulsions, and ataxia to paralysis. These effects were observed in 13-week studies of rats and dogs. Administration of 93 mg/kg/day 2,4-DNT in the diet for 13 weeks caused demyelination in the cerebellum and brain stem of 1 male rat, while at 266 mg/kg/day, some rats exhibited a widespread or stiff-legged gait that did not progress to the rigid paralysis observed in dogs (Lee et al. 1985; U.S. Army 1978b). After 3 months of being fed 2,4-DNT at 0.5% in the diet (an estimated dose of 350 mg/kg/day based on body weight and feed consumption data provided by the study authors), Wistar rats exhibited humpback and jerky incoordination (Kozuka et al. 1979). Dogs that were administered 25 mg/kg/day 2,4-DNT in capsules for 13 weeks began to show neurotoxic effects within 2 months; these effects included incoordination, abnormal gait, rigid paralysis of the hind legs, eventually

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progressing to paralysis up to the neck (Ellis et al. 1985; U.S. Army 1978b). No neurological signs were observed in mice fed 413 mg/kg/day in males or 468 mg/kg/day in females 2,4-DNT in the diet for 13 weeks (Hong et al. 1985; U.S. Army 1978b).

An abnormal gait was also observed in chronic studies of laboratory animals fed 2,4-DNT. The characteristic widespread and stiff-legged gait was observed after feeding 34.5 or 45.3 mg/kg/day 2,4-DNT to male and female rats, respectively, for up to 2 years (Lee et al. 1985; U.S. Army 1978b, 1979). This stiff-legged gait and hyperactive behavior were also noted in mice fed 898 mg/kg/day in the diet for 24 months but were not observed in mice at 95 mg/kg/day (Ellis et al. 1985; U.S. Army 1979). In dogs dosed at 1.5 mg/kg/day 2,4-DNT in a 2-year study, one of six dogs showed intermittent loss of hindquarter control (Ellis et al. 1985; U.S. Army 1979). Central nervous system lesions were identified in high-dose (10 mg/kg/day) dogs in this study and included vacuolization, hypertrophy, endothelial mitosis, and focal gliosis in the cerebellum, as well as some perivascular hemorrhage in the cerebellum and brain stem (Ellis et al. 1985; U.S. Army 1979).

Dogs dosed with 20 or 100 mg/kg/day of 2,6-DNT for 13 weeks exhibited dose-related neurotoxic symptoms that included muscular incoordination, weakness, tremors, and paralysis (U.S. Army 1976). Rats and mice dosed at 145 and 289 mg/kg/day of 2,6-DNT, respectively, for 13 weeks did not display neurotoxic symptoms (U.S. Army 1976).

Administration of 150 mg/kg/day Tg-DNT to F344 dams during gestation days 7–20 caused hindlimb weakness in 7 of 13 animals (Jones-Price et al. 1982). No clinical signs of neurotoxicity or histopathological changes were found in rats fed up to 35 mg/kg/day Tg-DNT in the diet for 26 or 52 weeks (Hazleton Laboratories 1982).

No studies were located regarding neurological effects in animals after oral exposure to 2,3-, 2,5-, or 3,5-DNT.

For 2,4-DNT, the highest NOAEL values and all LOAEL values from each reliable study for neurological effects in each species and duration category are recorded in [Table 3-1](#) and plotted in [Figure 3-1](#). For 2,6-DNT, the highest NOAEL values and from each reliable study for neurological effects in each species and duration category are recorded in [Table 3-2](#) and plotted in [Figure 3-2](#).

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3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Studies in laboratory animals have shown that oral exposure to 2,4-DNT can result in adverse effects on reproduction, as shown by decreased fertility and the development of lesions of the male and female reproductive tracts. The male reproductive system seems to be particularly sensitive; observed effects include decreased sperm production, testicular atrophy, changes in Sertoli cell morphology, and degenerated seminiferous tubules (Bloch et al. 1988; Kozuka et al. 1979; Lane et al. 1985; McGown et al. 1983; U.S. Army 1976, 1978b, 1979). In the female reproductive system, ovarian atrophy and dysfunction were observed (U.S. Army 1979).

The effects on the male reproductive system have been reported in studies of brief durations. Decreased fertility was noted in male rats dosed with 180 mg/kg 2,4-DNT for 5 days; no dominant lethal effect was observed at this dose (Lane et al. 1985). Sprague-Dawley rats administered 104, 165, or 261 mg/kg/day 2,4-DNT in the diet for 14 days exhibited oligospermia with degenerative changes, such as syncytial cell formation and focal spermatoc granuloma, in a dose-related manner (McGown et al. 1983). A concentration of 78 mg/kg/day 2,4-DNT caused a decrease in the thickness of spermatogenic cell layers. No histopathological changes were found in the reproductive organs of females in this study (McGown et al. 1983). Although no changes were found in sperm morphology of male mice that were administered 250 mg/kg/day 2,4-DNT for 2 days, significant decreases in fertile matings of these animals were observed during weeks 2, 3, and 6 post-treatment (Soares and Lock 1980). However, sperm morphology was examined at 8 weeks post-treatment, so it is possible that a toxic effect was selective for specific types of sperm cells.

In intermediate studies of 2,4-DNT, serious effects on the male reproductive system have been observed in numerous animal studies. In a series of three dominant lethal studies using male rats for 13 weeks, 45 mg/kg/day 2,4-DNT in the diet caused severe atrophy and degeneration of the seminiferous tubules, resulting in decreased fertility, although no dominant lethal effect was observed (U.S. Army 1979). Another study using CD rats found that spermatogenesis was impaired after 4 weeks of feeding 93 mg/kg/day 2,4-DNT in the diet and had completely ceased after 13 weeks (Lee et al. 1985; U.S. Army 1978b). This effect was not reversible after a 4-week post-treatment period. Higher concentrations of 2,4-DNT were needed to cause these effects in mice. Testicular atrophy and aspermatogenesis occurred

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in CD-1 mice fed 413 mg/kg/day 2,4-DNT for 13 weeks (Hong et al. 1985; U.S. Army 1978b) and rats fed a TWA dose of 371 mg/kg/day 2,4-DNT for 6 months in feed (Kozuka et al. 1979). Decreased fertility was observed after male mice were treated with 1,032 mg/kg/day, but not 295 mg/kg/day, 2,4-DNT in the feed for 4 weeks in a dominant lethal study (U.S. Army 1978b). The decreased fertility was not observed in mice fed 295 mg/kg/day (U.S. Army 1978b). The testicular atrophy was considered to be due to a direct toxic effect on spermatogenic cells. Mild-to-severe testicular degeneration with decreased spermatogenesis has also been observed in dogs administered 25 mg/kg 2,4-DNT in capsules for 13 weeks (Ellis et al. 1985; U.S. Army 1978b). No testicular effects were found at 5 mg/kg in the study.

Histopathological examination of the testes after treatment with 2,4-DNT revealed changes, which suggest specific causes for the male infertility observed in animal studies. Dose-related changes in sperm cell morphology were found in Sprague-Dawley rats fed 76.7 or 153.4 mg/kg/day 2,4-DNT in the diet for 3 weeks (Bloch et al. 1988). At the low dose, vacuolation and lipid accumulation were noted in Sertoli cells; multinucleated spermatid and irregularities of the basal lamina were also found. These changes were limited and variable with most samples, demonstrating patchy damage. More extensive degenerative changes in both spermatocytes and spermatids were found at the high dose as well as ultrastructural changes in Sertoli cells; epididymal sperm counts were decreased 63%. The high-dose animals also had increased levels of serum luteinizing hormone (LH) and FSH but not testosterone (Bloch et al. 1988).

Chronic-duration studies in laboratory animals have also demonstrated both male and female reproductive effects. Male CD-1 mice fed 14 mg/kg/day 2,4-DNT for 12 months showed atrophy of the testes and decreased spermatogenesis (Hong et al. 1985). Female mice fed 898 mg/kg/day 2,4-DNT in this study had ovarian atrophy with non-functioning follicles, with a NOAEL of 95 mg/kg/day (Hong et al. 1985). Male CD rats that received 34 mg/kg/day 2,4-DNT in the diet for 12 months showed an increased incidence of seminiferous tubule atrophy compared to controls (100% affected at the high dose versus 0% of controls), with a NOAEL of 3.9 mg/kg/day (Lee et al. 1985; U.S. Army 1978b, 1979). No adverse reproductive effects were found in dogs fed 10 mg/kg/day 2,4-DNT for 24 months (Ellis et al. 1985; U.S. Army 1979).

A three-generation reproductive toxicity study was performed in rats fed 2,4-DNT for up to 6 months before mating of original prenatal animals (U.S. Army 1979). Effects on neonatal viability were observed at the highest concentration of 2,4-DNT used, 40 mg/kg/day. Reductions in neonatal viability became

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more severe with successive litters within each generation, such that no second litters were produced by the second generation of high-dose animals, which were fed 34.5 mg/kg/day (male) or 45.3 mg/kg/day (female) 2,4-DNT. Decreased fetal viability was attributed to maternal neglect and maternal death during parturition. Decreases in the number of fetal implants were attributed to the adverse impact of 2,4-DNT on sperm production.

In studies of rats, mice, and dogs dosed with 2,6-DNT for 13 weeks (U.S. Army 1976), decreased spermatogenesis was observed in male mice administered 51 mg/kg/day, but normal spermatogenesis was observed in animals dosed with 11 mg/kg/day. Testicular atrophy was reported in rats administered 35 mg/kg/day 2,6-DNT, and no effects were observed in rats dosed with 7 mg/kg/day (U.S. Army 1976). Dogs dosed with 20 and 100 mg/kg/day had testicular degeneration, but no effects were observed in dogs dosed with 4 mg/kg/day (U.S. Army 1976).

Based upon the testicular effects observed after administration of 2,4- or 2,6-DNT, it is not surprising that these effects are found after treatment with Tg-DNT. Testicular degeneration was found in male rats fed 35 mg/kg/day Tg-DNT for 26 weeks, but since the finding was unilateral, the relationship to treatment may be considered equivocal (Hazleton Laboratories 1982). When treatment with this concentration was carried through 52 weeks, however, bilateral mild-to-severe testicular degeneration and hypospermatogenesis were observed (Hazleton Laboratories 1982). No changes were found in the fertility or sperm morphology of male mice that received 250 mg/kg Tg-DNT by gavage for 2 days in a dominant lethal study (Soares and Lock 1980).

No studies were located regarding reproductive effects in animals after oral exposure to 2,3-, 2,5-, or 3,5-DNT.

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in [Tables 3-1](#) and [3-2](#) and plotted in [Figures 3-1](#) and [3-2](#).

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT. However, developmental toxicity from DNT could potentially occur because exposure to any substance that depletes the amount of oxygen available to developing fetal tissues can have adverse consequences. U.S. Army (1979) conducted a 3-generation reproductive study in which 2,4-DNT was

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administered to male and female rats at doses up to 34.5 and 45.3 mg/kg/day, respectively. Normal birth weights, liveborn index, and weight at weaning were observed. Decreases in pup viability at 45.3 mg/kg/day in this study resulted from maternal neglect and a high incidence of maternal death during parturition; these decreases did not appear to result from pup defects since no anomalies were detected in offspring from any generation. These effects were not observed in animals fed 5.1 mg/kg/day 2,4-DNT (U.S. Army 1979).

Tg-DNT was administered by gavage to pregnant rats for 14 days during gestation, and pups were evaluated for developmental toxicity either at gestation day 20 or postpartum day 60 (Jones-Price et al. 1982). Adverse effects on hematologic parameters and altered organ weights were observed in both dams and fetuses when dams were administered 100 or 150 mg/kg/day. However, the fetal toxicity was not dose related. A decrease in relative liver weight was observed, however, in the postpartum pups at the low dose of 14 mg/kg/day; this dose is considered to be a LOAEL. Dose-related effects on postnatal development were not observed in pups when dams were administered 35 or 75 mg/kg/day. Transient and statistically significant signs of neurotoxicity, which were not dose-related, included delayed eye opening and cliff avoidance when dams were treated with 35 or 75 mg/kg/day. No evidence of toxicity was found in pups at postpartum day 60 of the postnatal study.

No studies were located regarding developmental effects in animals after oral exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category are recorded in [Tables 3-1](#) and [3-2](#) and plotted in [Figures 3-1](#) and [3-2](#).

3.2.2.7 Cancer

No studies were located regarding cancer in humans after oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

The carcinogenic activity of DNT has been extensively studied in typical chronic bioassays and in some less-than-lifetime studies. 2,4-DNT produced renal tumors in male mice and was hepatocarcinogenic in rats. 2,6-DNT and Tg-DNT are potent hepatocarcinogens in rats (Lee et al. 1985; U.S. Army 1978b, 1979).

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2,4-DNT (98% 2,4-DNT, 2% 2,6-DNT) produced renal tumors (76%) in male CD-1 mice fed 95 mg/kg/day for 2 years (U.S. Army 1979). A statistically significant increase in renal tumors in female mice was not observed. A National Cancer Institute (NCI) bioassay (NCI 1978) of 2,4-DNT (95% 2,4-DNT, the other components not specified) did not detect a carcinogenic effect in mice dosed with 72 mg/kg/day for 78 weeks. The NCI bioassay used the C57BL/6N strain of mouse, lower doses, and a shorter treatment schedule than did U.S. Army (1979).

Hepatocellular carcinoma were significantly increased in male CD rats fed 34.5 mg/kg/day 2,4-DNT and in females fed 45.3 mg/kg/day 2,4-DNT for 2 years (U.S. Army 1979). The tumor response in females was higher than in the males. Two other studies of rats in which malignancies were not observed used the F344 strain, lower doses, and shorter exposure durations than did U.S. Army (1979): 10 mg/kg/day for 78 weeks (NCI 1978) and 27 mg/kg/day for 52 weeks (Leonard et al. 1987). NCI (1978) reported significant increases in subcutaneous tissue fibroma in male rats at 7.5–8 mg/kg/day and mammary gland fibroadenomas in female rats at 22 mg/kg/day. U.S. Army (1979) found significant increases in subcutaneous tissue fibromas in male rats at 34.5 mg/kg/day and mammary gland fibroadenomas in female rats at 45.3 mg/kg/day; these were benign tumors.

2,4-DNT was not found to be carcinogenic in the Strain A/J mouse pulmonary tumor bioassay when 250 mg/kg was administered by gavage twice a week for 12 weeks (Stoner et al. 1984). 2,4-DNT was a hepatic tumor promoter, but not a tumor initiator, using *in vivo* hepatic initiation-promotion protocols (Leonard et al. 1986).

2,6-DNT administered for 1 year at 7 and 14 mg/kg/day produced hepatocellular carcinomas in 85% and 100%, respectively, of male F344 rats (Leonard et al. 1987). Pulmonary metastases of hepatocytic origin were also observed. Both tumor-initiating and tumor-promoting activities of 2,6-DNT in rat liver were reported (Leonard et al. 1983, 1986; Mirsalis and Butterworth 1982). 2,6-DNT was not found to be a lung carcinogen in the Strain A/J mouse pulmonary tumor bioassay when 250 mg/kg was administered by gavage twice a week for 12 weeks (Schut et al. 1983; Stoner et al. 1984).

The effect of diet-induced changes in gut microflora on the hepatocarcinogenicity of 2,6-DNT was studied in male F344 rats (Goldsworthy et al. 1986). Groups of the rats were placed on one of three diets containing 2,6-DNT at doses of 0, 0.6–0.7, or 3–3.5 mg/kg/day. Ten animals from each group were sacrificed at 3, 6, and 12 months, and the livers were evaluated histopathologically. The diets used were NIH-07, an open formula cereal-based diet high in pectin content; AIN-76A, a purified pectin-free diet; or

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AP, which is AIN-76A supplemented with 5% pectin. The number and size of γ -glutamyl transpeptidase-staining foci in the liver increased in a dose- and time-related manner in animals given 2,6-DNT in the NIH-07 diet. Hepatocellular carcinomas and neoplastic nodules were observed only in rats fed NIH-07 containing 2,6-DNT. No tumor was observed in rats receiving the control diets or 2,6-DNT in the AIN-76 diet with or without pectin. This finding suggested that pectin did not influence the tumor outcome of the experiment. Unidentified contaminants in cereal-based diets may influence liver foci and tumor production in the rat liver during carcinogen treatment.

Tg-DNT provided positive hepatocarcinogenic results in two bioassays of less-than-lifetime duration. In a 52-week study of male rats dosed with 35 mg/kg/day of Tg-DNT, Leonard et al. (1987) observed a 47% increase in hepatocellular carcinoma; cholangiocarcinomas were also found in 10% of rats treated with 35 mg/kg/day Tg-DNT in the Leonard et al. (1987) study. Hazleton Laboratories (1982) reported that dietary administration of 35 mg/kg/day Tg-DNT to rats for 55 weeks resulted in an increased incidence (100% in males and 55% in females) of hepatocellular carcinoma; this lesion was found in some animals treated at this level for 26 weeks. The administration of 3.5 mg/kg/day Tg-DNT for 104 weeks caused hepatocellular carcinoma in 9 of 70 males compared to 1 of 61 controls. Mammary fibroadenoma and subcutaneous fibroma were also found in both sexes at 3.5 mg/kg/day after 104 weeks (Hazleton Laboratories 1982). Administration of 14 mg/kg/day Tg-DNT for 104 weeks caused cholangiocarcinomas and parathyroid adenomas in males and hepatocellular carcinomas and hepatocholangiocarcinomas in females (Hazleton Laboratories 1982).

Tg-DNT contains about 76% 2,4-DNT and 19% 2,6-DNT, as well as small amounts of other isomers. Rats that received 35 mg/kg/day in the Leonard et al. (1987) and Hazleton Laboratories (1982) studies were provided approximately 28 and 7 mg/kg/day of 2,4- and 2,6-DNT, respectively. This dose of 2,6-DNT in Tg-DNT bioassays is equivalent to the low dose of 2,6-DNT administered to rats by Leonard et al. (1987) that produced hepatocellular carcinomas.

In hepatic tumor initiation-promotion protocols, Tg-DNT was reported to have tumor-promoting and tumor-initiating activity (Leonard et al. 1983, 1986; Mirsalis and Butterworth 1982). The results of the initiation-promotion protocols for 2,4-, 2,6-, and Tg-DNT indicate that 2,6-DNT is a complete hepatocarcinogen and is primarily responsible for the carcinogenic activity of Tg-DNT.

No studies were located regarding cancer in animals after oral exposure to 2,3-, 2,5-, or 3,5-DNT.

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3.2.3 Dermal Exposure

There are no data regarding adverse health effects associated with dermal exposure of humans or animals to 2,3-, 2,5-, or 3,5-DNT. There are data on occupational exposure of humans to 2,4-DNT and Tg-DNT (see Section 3.2.1) in which dermal exposure probably occurred, but the primary route of exposure in these studies is believed to be inhalation. The relative contribution of dermal exposure to total occupational exposure cannot be determined from these studies. Levine et al. (1985b) reported that small amounts of 2,4-DNT were detected on the hands, face, and forehead when a wipe-sample survey was conducted on workers in a DNT manufacturing plant. The highest quantity found on a worker's skin was 180 µg and may account for the quantity of excreted urinary metabolites that exceeded the amount of inhaled DNT in the operators and loaders. Animal data associated with dermal exposure of animals to DNT are limited to studies of dermal irritation, eye irritation, and dermal sensitization of 2,4-, 2,6-, or Tg-DNT in rabbits.

3.2.3.1 Death

No studies were located regarding mortality in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

One study was located that examined death among humans exposed to DNT. A retrospective mortality study of munitions workers exposed to either 2,4-DNT or Tg-DNT revealed an increased death rate due to ischemic heart disease and residual diseases of the circulatory system in the exposed cohort (Levine et al. 1986a, 1986b). Exposure concentrations to 2,4-DNT or Tg-DNT were not reported. The residual diseases included cardiac arrest and arteriosclerosis. Exposure levels were not reported, and the study is further limited by the small cohort size and concurrent inhalation exposure of the workers.

No studies were located regarding death in animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, renal, body weight, or endocrine effects in humans or animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

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Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

Gastrointestinal complaints of munitions workers exposed to either 2,4-DNT or Tg-DNT included nausea and vomiting (McGee et al. 1947). These workers also presumably inhaled DNT in the occupational setting. Exposure concentrations to 2,4-DNT or Tg-DNT were not reported.

No studies were located regarding gastrointestinal effects in animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Cardiovascular Effects. No studies were identified with respect to cardiovascular effects after dermal exposure of humans to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

Levine et al. (1986a) reported a significant increase in heart disease mortality in workers involved in the manufacture and processing of 2,4-DNT and/or Tg-DNT.

No studies were located regarding cardiovascular effects in animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Hematological Effects. No studies were located regarding hematological effects in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

Hematological effects, such as anemia and cyanosis (which can be indicative of anemia), have been found in men employed at munitions factories (McGee et al. 1947; Perkins 1919). These workers were exposed to either 2,4-DNT or Tg-DNT. Because these studies lacked worker histories, exposure data, and reported on small cohorts, the results are equivocal and are best used to qualitatively describe symptoms. In addition, the workers probably received their primary exposure via the inhalation pathway.

No studies were located regarding hematological effects in animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

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Muscle weakness and joint pain have been reported by munitions workers after occupational exposure to unspecified concentrations of 2,4-DNT or Tg-DNT (McGee et al. 1947; Perkins 1919).

In the Perkins (1919) study, joint pain and other incapacitating symptoms were noted following exposure to what were presumed to be very high concentrations of Tg-DNT since the processes described required direct handling without protective equipment. In both of these studies, however, no exposure data were available; exposure to other compounds may have occurred, and concomitant exposure via inhalation was also likely.

No studies were located regarding musculoskeletal effects in animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Hepatic Effects. No studies were located regarding hepatic effects in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

In a study of male munitions workers exposed to unspecified concentrations of 2,4-DNT, 29 of 714 workers displayed tenderness of the liver (McGee et al. 1947). No other clinical evaluation was performed that might provide further insight into the significance of this finding. These workers were also exposed to DNT via inhalation.

No studies were located regarding hepatic effects in animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Dermal Effects. No studies were located regarding dermal effects in humans associated with dermal exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

A study of dermal effects of topical exposure to 2,4-DNT in workers employed by a munitions factory during World War II reported that 32 of 714 workers complained of dermatitis (McGee et al. 1947). Exposure levels were not quantified in this study.

Both 2,4- and 2,6-DNT were shown to be mild primary dermal irritants in rabbits (U.S. Army 1975, 1978a).

3. HEALTH EFFECTS

No studies were located regarding dermal effects in animals after dermal exposure to 2,3-, 2,5-, or 3,5-DNT.

Ocular Effects. No studies were located regarding ocular effects in humans after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

No ocular irritation was found in rabbits in a primary eye irritation test using unspecified concentrations of 2,4- or 2,6-DNT (U.S. Army 1975, 1978a). However, mild eye irritations were reported in rabbits treated with 2,4-DNT (Ford 1981).

No studies were located regarding ocular effects in animals after dermal exposure to 2,3-, 2,5-, or 3,5-DNT.

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

In dermal sensitization tests, 2 of 10 guinea pigs exhibited mild sensitization to 2,6-DNT, but no sensitization was evident when 2,4-DNT was tested (U.S. Army 1975, 1978a).

No studies were located regarding immunological and lymphoreticular effects in animals after dermal exposure to 2,3-, 2,5-, or 3,5-DNT.

3.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans after dermal exposure to 2,3-, 2,5-, 2,6-, or 3,5-DNT.

Various neurological symptoms, including headache, vertigo, and pain or numbness in the extremities, have been reported in surveys of munitions workers exposed to unspecified concentrations of 2,4-DNT (McGee et al. 1947). Although it is assumed that some dermal exposure to 2,4-DNT occurred in these workers, inhalation was the probable primary route of exposure.

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No studies were located regarding neurological effects in animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

3.2.3.5 Reproductive Effects

No significant effects on fertility were observed in workers occupationally exposed to Tg-DNT (Levine et al. 1985a). However, Levine et al. estimated that only a 50–70% reduction in fertility could have been detected in the worker population that they studied.

No studies were located regarding reproductive effects in animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

No studies were located regarding the following health effects in humans or animals after dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT:

3.2.3.6 Developmental Effects**3.2.3.7 Cancer****3.3 GENOTOXICITY**

Data are available for 2,3-, 2,4-, 2,5-, 2,6-, 3,5-, and Tg-DNT from *in vitro* tests in prokaryotic organisms (gene mutation in *Salmonella typhimurium*) and in mammalian cell systems (gene mutation, chromosomal aberrations, DNA damage, and morphological transformation). Results of *in vitro* genotoxicity assays are presented in [Table 3-3](#). *In vivo* data for 2,3-, 2,4-, 2,5-, 2,6-, 3,5-, and Tg-DNT, including evaluations of unscheduled DNA synthesis (UDS), DNA damage and DNA binding, gene mutations, and/or chromosomal aberrations, are presented in [Table 3-4](#). Some isomers of DNT (2,4-DNT and especially 2,6-DNT) have tested positive *in vitro* and/or *in vivo* in other genotoxicity assays, with evidence of chromosomal aberrations, DNA damage and adduct formation, and morphological transformation. 2,3-, 2,5-, and 3,5-DNT generally tested negative in genotoxicity assays. Taken together, results of *in vitro* studies do not provide strong evidence that DNT directly induces gene mutations; however, there is some evidence that 2,4-DNT induces mutations *in vivo*.

In vitro studies assessing genotoxicity of DNT have yielded mixed results in *S. typhimurium*, presumably due to differences in the need for metabolic activation and the sensitivity of the tester strains. 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT have been shown to induce gene mutations in *S. typhimurium* in the presence or

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Table 3-3. Genotoxicity of DNT Isomers *In Vitro*

Species (test system)	Isomer	End point	Results		Reference
			With activation	Without activation	
Prokaryotic organisms:					
<i>Salmonella typhimurium</i> , TA98	2,3-DNT	Gene mutation	+	+	Couch et al. 1981
<i>S. typhimurium</i> , TM677	2,3-DNT	Gene mutation	–	–	Couch et al. 1981
<i>S. typhimurium</i> , TA98, TA100	2,3-DNT	Gene mutation	–	–	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA98; with flavin mononucleotide (FMN)	2,3-DNT	Gene mutation (modified assay)	+	NT	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA100; with FMN	2,3-DNT	Gene mutation (modified assay)	–	NT	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA100	2,3-DNT	Gene mutation	+	+	Spanggord et al. 1982b
<i>S. typhimurium</i> , TA1538	2,3-DNT	Gene mutation	–	+	Spanggord et al. 1982b
<i>S. typhimurium</i> , TA1535, TA1537, TA100 NR3	2,3-DNT	Gene mutation	–	–	Spanggord et al. 1982b
<i>S. typhimurium</i> , TA100	2,3-DNT	Gene mutation	±	–	Kawai et al. 1987
<i>S. typhimurium</i> , TA98	2,3-DNT	Gene mutation	–	±	Kawai et al. 1987
<i>S. typhimurium</i> , TA98, TM677	2,4-DNT	Gene mutation	+	+	Couch et al. 1981
<i>S. typhimurium</i>	2,4-DNT	Gene mutation	NT	–	Chiu et al. 1978
<i>S. typhimurium</i>	2,4-DNT	Gene mutation	+	+	Tokiwa et al. 1981
<i>S. typhimurium</i> , TA98, TA100	2,4-DNT	Gene mutation	–	–	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA98; with FMN	2,4-DNT	Gene mutation (modified assay)	+	NT	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA100; with FMN	2,4-DNT	Gene mutation (modified assay)	–	NT	Dellarco and Prival 1989
<i>S. typhimurium</i>	2,4-DNT	Gene mutation	NT	+	Mori et al. 1982
<i>S. typhimurium</i> , TA100	2,4-DNT	Gene mutation	–	+	Spanggord et al. 1982b
<i>S. typhimurium</i> , TA100 NR3	2,4-DNT	Gene mutation	+	–	Spanggord et al. 1982b
<i>S. typhimurium</i> , TA1535, TA1537, TA1538, TA98	2,4-DNT	Gene mutation	–	–	Spanggord et al. 1982b
<i>S. typhimurium</i> , TA100	2,4-DNT	Gene mutation	+	–	U.S. Army 1978a
<i>S. typhimurium</i> , TA1535	2,4-DNT	Gene mutation	–	+	U.S. Army 1978a
<i>S. typhimurium</i> , TA98, 1537	2,4-DNT	Gene mutation	–	–	U.S. Army 1978a
<i>S. typhimurium</i> , TA1538	2,4-DNT	Gene mutation	+	+	U.S. Army 1978a

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Table 3-3. Genotoxicity of DNT Isomers *In Vitro*

Species (test system)	Isomer	End point	Results		Reference
			With activation	Without activation	
<i>S. typhimurium</i>	2,4-DNT	Gene mutation	+	NT	Pearson et al. 1978
<i>S. typhimurium</i> , TA98, TA98 NR, TA98/8-DNP ₆ , YG1021, YG1024	2,4-DNT	Gene mutation	NT	+	Einistö et al. 1991
<i>S. typhimurium</i> , TA100	2,4-DNT	Gene mutation	±	±	Kawai et al. 1987
<i>S. typhimurium</i> , TA98	2,4-DNT	Gene mutation	–	–	Kawai et al. 1987
<i>S. typhimurium</i> , TA100	2,4-DNT	Gene mutation	NT	–	Padda et al. 2003
<i>S. typhimurium</i> , TA98,	2,4-DNT	Gene mutation	NT	–	Sayama et al. 1998
<i>S. typhimurium</i> , TA100, YG1021, YG1024, YG1041, YG1026, YG1029, YG1042	2,4-DNT	Gene mutation	NT	+	Sayama et al. 1998
<i>Escherichia coli</i>	2,4-DNT	Gene mutation	–	–	Dunkel et al. 1985
<i>S. typhimurium</i> , NM2009, NM3009	2,4-DNT	DNA damage (umu test)	NT	+	Öztürk and Durusoy 1999
<i>E. coli</i> , PQ37	2,4-DNT	DNA damage (SOS – chromotest)	–	–	Öztürk and Durusoy 1999
<i>S. typhimurium</i> , TA98, TM677	2,5-DNT	Gene mutation	+	+	Couch et al. 1981
<i>S. typhimurium</i> , TA98	2,5-DNT	Gene mutation	±	No data	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA100	2,5-DNT	Gene mutation	–	–	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA98; with FMN	2,5-DNT	Gene mutation (modified assay)	+	NT	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA100; with FMN	2,5-DNT	Gene mutation (modified assay)	–	NT	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA1535, TA1538, TA98, TA100	2,5-DNT	Gene mutation	+	+	Spanggord et al. 1982b
<i>S. typhimurium</i> , TA1537, TA100 NR3	2,5-DNT	Gene mutation	–	–	Spanggord et al. 1982b
<i>S. typhimurium</i> , TA98, TM677	2,6-DNT	Gene mutation	+	+	Couch et al. 1981
<i>S. typhimurium</i> , TA100, TA1535, TA1537, TA98	2,6-DNT	Gene mutation	–	–	U.S. Army 1978a
<i>S. typhimurium</i> , TA1538	2,6-DNT	Gene mutation	–	+	U.S. Army 1978a
<i>S. typhimurium</i>	2,6-DNT	Gene mutation	NT	+	Simmon et al. 1977
<i>S. typhimurium</i> , TA98, TA100	2,6-DNT	Gene mutation	–	–	Sayama et al. 1989b
<i>S. typhimurium</i>	2,6-DNT	Gene mutation	–	+	Tokiwa et al. 1981
<i>S. typhimurium</i> , TA100	2,6-DNT	Gene mutation	+	+	Spanggord et al. 1982b

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Table 3-3. Genotoxicity of DNT Isomers *In Vitro*

Species (test system)	Isomer	End point	Results		Reference
			With activation	Without activation	
<i>S. typhimurium</i> , TA1535, TA1537, TA1538, TA98, TA100 NR3	2,6-DNT	Gene mutation	–	–	Spanggord et al. 1982b
<i>S. typhimurium</i> , TA98, TA100	2,6-DNT	Gene mutation	–	–	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA98; with FMN	2,6-DNT	Gene mutation (modified assay)	+	NT	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA100; with FMN	2,6-DNT	Gene mutation (modified assay)	–	NT	Dellarco and Prival 1989
<i>S. typhimurium</i> , TA98, TA100, TA1537	2,6-DNT	Gene mutation	+	+	Suzuki et al. 2011
<i>S. typhimurium</i> , TA98, TA98/1,8-DNP, YG1021, YG1024	2,6-DNT	Gene mutation	NT	+	Einistö et al. 1991
<i>S. typhimurium</i> , TA98 NR	2,6-DNT	Gene mutation	+	–	Einistö et al. 1991
<i>S. typhimurium</i>	2,6-DNT	Gene mutation	+	NT	Pearson et al. 1979
<i>S. typhimurium</i> , TA98, TA100	2,6-DNT	Gene mutation	±	±	Kawai et al. 1987
<i>S. typhimurium</i> , TA100	2,6-DNT	Gene mutation	NT	–	Padda et al. 2003
<i>S. typhimurium</i> , TA98, TA100 YG1021,	2,6-DNT	Gene mutation	NT	–	Sayama et al. 1998
<i>S. typhimurium</i> , YG1024, YG1041, YG1026, YG1029, YG1042	2,6-DNT	Gene mutation	NT	+	Sayama et al. 1998
<i>S. typhimurium</i> , TA98, TM677	3,5-DNT	Gene mutation	+	+	Couch et al. 1981
<i>S. typhimurium</i> , TA1537, TA1538, TA98, TA100	3,5-DNT	Gene mutation	+	+	Spanggord et al. 1982b
<i>S. typhimurium</i> , TA1535, TA100 NR3)	3,5-DNT	Gene mutation	–	–	Spanggord et al. 1982b
<i>S. typhimurium</i> , TA98, TM677	Tg-DNT	Gene mutation	+	+	Couch et al. 1981
<i>S. typhimurium</i>	Tg-DNT	Gene mutation	NT	+	Chadwick et al. 1990
Mammalian cells:					
CHO cells	2,4-DNT	Sister chromatid exchange	+	–	Loveday et al. 1989
CHO cells	2,4-DNT	Chromosomal aberrations	–	–	Loveday et al. 1989
CHO/HGPRT	2,3-DNT	Gene mutation	–	–	Abernethy and Couch 1982
CHO/HGPRT	2,4-DNT	Gene mutation	–	–	Abernethy and Couch 1982

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Table 3-3. Genotoxicity of DNT Isomers *In Vitro*

Species (test system)	Isomer	End point	Results		Reference
			With activation	Without activation	
CHO/HGPRT	2,4-DNT	Gene mutation	–	–	Abernethy and Couch 1982
CHO/HGPRT	2,5-DNT	Gene mutation	–	–	Abernethy and Couch 1982
CHO-HGPRT	2,6-DNT	Gene mutation	–	–	Abernethy and Couch 1982
CHO/HGPRT	3,5-DNT	Gene mutation	–	–	Abernethy and Couch 1982
CHO-HGPRT	Tg-DNT	Gene mutation	–	–	Abernethy and Couch 1982
P388 mouse lymphoma TK	2,4-DNT	Gene mutation	–	+	Styles and Cross 1983
P388 mouse lymphoma TK	2,6-DNT	Gene mutation	–	–	Styles and Cross 1983
P388 mouse lymphoma TK	Tg-DNT	Gene mutation	–	–	Styles and Cross 1983
Chinese hamster lung fibroblasts	2,6-DNT	Chromosomal aberrations	+	+	Suzuki et al. 2011
SHE cells	2,4-DNT	Morphological transformation	NT	–	Holen et al. 1990
SHE cells	2,4-DNT	Morphological transformation	NT	+	Engelhardt et al. 2004
SHE cells	2,6-DNT	Morphological transformation	NT	+	Engelhardt et al. 2004
SHE cells	2,6-DNT	Morphological transformation	NT	–	Holen et al. 1990
Kumming rat Sertoli cells	2,4-DNT	DNA damage (comet assay)	NT	+	Yang et al. 2005
Kumming rat Sertoli cells	2,6-DNT	DNA damage (comet assay)	NT	+	Yang et al. 2005
Human peripheral lymphocytes	2,4-DNT	Chromosomal aberrations	NT	+	Huang et al. 1996

+ = positive; – = negative; ± = weakly positive or equivocal; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; DNT = dinitrotoluene; FMN = flavin mononucleotide; NT = not tested; SHE = Syrian hamster embryo; Tg = technical grade

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Table 3-4. Genotoxicity of DNT Isomers *In Vivo*

Species (test system)	Isomer	End point	Results	Reference
DNT and TNT workers	Tg-DNT	Chromatid-type chromosomal aberrations	+	Sabbioni et al. 2006
Rat hepatocyte	2,4-DNT	UDS	+	Mirsalis et al. 1989
Rat hepatocyte	2,4-DNT	UDS	+	Mirsalis and Butterworth 1982
Rat hepatocyte (male; AP or F344)	2,4-DNT ^a	UDS	+	Ashby et al. 1985
Rat hepatocyte	2,4-DNT	S-phase synthesis	+	Mirsalis et al. 1989
Rat hepatocyte (male; Sprague-Dawley)	2,3-DNT	DNA damage; comet assay	–	Lent et al. 2012
Rat hepatocyte (male; Sprague-Dawley)	2,4-DNT	DNA damage; comet assay	–	Lent et al. 2012
Rat hepatocyte (male; Sprague-Dawley)	2,5-DNT	DNA damage; comet assay	–	Lent et al. 2012
Rat hepatocyte (male; Sprague-Dawley)	2,6-DNT	DNA damage; comet assay	+	Lent et al. 2012
Rat hepatocyte (male; Crl:CD(SD))	2,6-DNT	DNA damage; comet assay	+	Suzuki et al. 2011
Rat hepatocyte (male; Sprague-Dawley)	3,5-DNT	DNA damage; comet assay	–	Lent et al. 2012
Rat hepatocyte	2,4-DNT	DNA binding (adduct formation)	+	La and Froines 1993
Rat hepatocyte (male; F344)	2,4-DNT	DNA binding (adduct formation)	+	Chadwick et al. 1993
Rat hepatocyte (female; Wistar)	2,4-DNT	DNA binding (adduct formation)	+	Jones et al. 2005a
Rat hepatocyte (female; Wistar)	2,6-DNT	DNA binding (adduct formation)	+	Jones et al. 2005a
Rat (male; F344) and mouse (male; B6C3F1) hepatocytes	2,6-DNT	DNA binding (adduct formation)	+	George et al. 1996
Rat peripheral blood (male; Sprague-Dawley)	2,3-DNT	Chromosomal aberrations; micronucleus assay	–	Lent et al. 2012
Rat hepatocyte (male; F344)	2,4-DNT	Chromosomal aberrations; micronucleus assay	+	Suzuki et al. 2009
Rat hepatocyte (male; F344)	2,4-DNT	Chromosomal aberrations; micronucleus assay	+	Takasawa et al. 2010
Rat peripheral blood (male; Sprague-Dawley)	2,4-DNT	Chromosomal aberrations; micronucleus assay	–	Lent et al. 2012
Rat peripheral blood (male; Sprague-Dawley)	2,5-DNT	Chromosomal aberrations; micronucleus assay	–	Lent et al. 2012
Rat peripheral blood (male; Sprague-Dawley)	2,6-DNT	Chromosomal aberrations; micronucleus assay	–	Lent et al. 2012
Rat hepatocyte (male; F344)	2,6-DNT	Chromosomal aberrations; micronucleus assay	±	Takasawa et al. 2010

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Table 3-4. Genotoxicity of DNT Isomers *In Vivo*

Species (test system)	Isomer	End point	Results	Reference
Rat peripheral blood (male; Sprague-Dawley)	3,5-DNT	Chromosomal aberrations; micronucleus assay	–	Lent et al. 2012
Mouse bone marrow (male; CBA x BALBc)F1)	2,4-DNT ^a	Chromosomal aberrations; micronucleus assay	–	Ashby et al. 1985
Mouse (female; C57BL/6J or T stock)	2,4-DNT ^a	Gene mutation, spot test	–	Soares and Lock 1980

^aThe test substance is referred to in the publication as technical-grade 2,4-DNT.

+ = positive result; – = negative result; ± = equivocal result; DNA = deoxyribonucleic acid; DNT = dinitrotoluene; Tg = technical grade; TNT = trinitrotoluene; UDS = unscheduled DNA synthesis

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absence of metabolic activation in some assays, but produced negative or equivocal results in several others (Couch et al. 1981; Dellarco and Prival, 1989; Einistö et al. 1991; Kawai et al. 1987; Mori et al. 1982; Neuwoehner et al. 2007; Padda et al. 2003; Sayama et al. 1989b; Simmon et al. 1977; Spanggord et al. 1982b; Suzuki et al. 2011; Tokiwa et al. 1981; U.S. Army 1978a). Sayama et al. (1998) showed that 2,4-DNT was mutagenic in *S. typhimurium* strains possessing high levels of nitroreductase and *O*-acetyltransferase and 2,6-DNT was mutagenic in strains possessing high levels of *O*-acetyltransferase. 2,4-DNT was nonmutagenic in *Escherichia coli* (Dunkel et al. 1985). Mixed results were found for 1,4-DNT in tests of DNA damage in bacteria; positive results were found in the umu test using *S. typhimurium* and negative results were found in the SOS chromotest using *E. coli* (Öztürk and Durusoy 1999). In general, 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT did not induce gene mutations in mammalian cells (Abernathy and Couch 1982; Styles and Cross 1983); however, positive results were obtained for 2,4-and/or 2,6-DNT in assays that evaluated chromosomal aberrations in Chinese hamster lung fibroblasts or human peripheral lymphocytes (Huang et al. 1996; Suzuki et al. 2011), morphological transformation in Syrian hamster embryo cells (Engelhardt et al. 2004; Holen et al. 1990), or DNA damage in rat Sertoli cells (Yang et al. 2005).

UDS and/or S-phase synthesis (SPS) were induced *in vitro* in the hepatocytes of F344 rats treated with 2,4-DNT or Tg-DNT *in vivo* (Ashby et al. 1985; Mirsalis et al. 1989). The mutagenicity of several of the metabolites of 2,6-DNT have been tested in *S. typhimurium*. Although neither 2,6-DNT nor its metabolites, 2-amino-6-nitrotoluene, 2,6-dinitrobenzylalcohol, 2-acetylamino-6-nitrobenzoic acid, and 2-amino-6-nitrobenzoic acid, were mutagenic in this assay with or without S9 activation, other metabolites of 2,6-DNT were found to possess mutagenic activity (Sayama et al. 1989b). The putative metabolite 2,6-dinitrobenzaldehyde was a direct acting mutagen (i.e., it did not require activation) (Sayama et al. 1989b). Urine from F344 rats administered 75 mg/kg 2,6-DNT by gavage tested positive for mutagenicity using *S. typhimurium* TA98 without S9 activation (Chadwick et al. 1993).

2,4-DNT induced lethal mutations but not reciprocal translocations in mutagenicity testing using *Drosophila melanogaster* (Woodruff et al. 1985). Color coat mutations were also observed in the offspring of pregnant C57BL/6J or T stock mice administered 1,000 mg/kg technical-grade 2,4-DNT via gavage or intraperitoneal injection on gestation day 10 (Soares and Lock 1980).

Micronuclei formation was observed in two assays in liver cells of 2,4-DNT-treated F344 rats (Suzuki et al. 2009; Takasawa et al. 2010), but were not observed in the peripheral blood of 2,4-DNT-treated Sprague-Dawley rats (Lent et al. 2012). Similarly, no micronuclei were observed in the peripheral blood

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of Sprague-Dawley rats administered 2,6-DNT via gavage for 2 or 14 days (Lent et al. 2012; Takasawa et al. 2010); however, micronuclei were induced in the hepatocytes of 2,6-DNT-treated rats. Other isomers of DNT (2,3-, 2,5-, or 3,5-DNT) tested negative in micronucleus assays in rats (Lent et al. 2012). In workers at a DNT and TNT manufacturing facility, significant increases in chromatid-type chromosomal aberrations were observed, as compared to factory controls (Sabbioni et al. 2006). When workers were divided into three age categories, a significant association between 2,4-DNT hemoglobin adducts and chromosomal frequency was observed in the youngest group, but not in the two older groups.

The formation of DNA adducts is generally thought to indicate carcinogenic risk (La and Froines 1993). Both 2,4- and 2,6-DNT have induced DNA adducts in rat liver. Following treatment with 2,4-DNT at up to 375 mg/kg via intraperitoneal injection, three DNA adducts were found in the liver of F344 rats (La and Froines 1992). Four adducts, identified as 4-amino-2-nitrotoluene (4A2NT), 2,4-TDA, and 4-acetylamino-2-aminotoluene (4AA2AT) (with 4A2NT being the predominant form) were found in the liver of Wistar rats administered 0.5 mmol/kg (approximately 91 mg/kg) 2,4-DNT as a single gavage dose (Jones et al. 2005a). Following treatment with 2,6-DNT, four DNA adducts (not identified) were found in the liver of rats treated with 75 mg/kg 2,6-DNT by gavage (Chadwick et al. 1993). In Wistar rats treated with 2,6-DNT at 0.5 mmol/kg (about 91 mg/kg) via gavage as a single dose, three adducts, identified as 2-amino-6-nitrotoluene (2A6NT), 2,6-TDA, and 2-acetylamino-6-aminotoluene (2AA6AT) (with 2A6NT being the predominant form) were detected (Jones et al. 2005a). Two types of DNA adducts were detected in liver DNA of B6C3F1 mice administered 2,6-DNT at 50 mg/kg via gavage; these adducts differed from the four types of adducts identified in F344 rats administered 2,6-DNT via gavage (George et al. 1996). The formation of 4 DNA adducts was also observed after intraperitoneal administration of 219 mg/kg 2,6-DNT to F344 rats (La and Froines 1992, 1993). One adduct accounts for the majority of the radioactivity measured; about 85% of the total was in one adduct in the study using 2,4-DNT, while in the study with 2,6-DNT, about 60% of the total adducts measured were from a single adduct with the other adducts constituting 10–15% of the total (La and Froines 1992, 1993). No quantitative or qualitative differences in adduct formation were found when treatment occurred by gavage or intraperitoneal injection (La and Froines 1992). The proximate DNA binding species has been postulated to be 2-hydroxylamino-6-nitrobenzyl alcohol (La and Froines 1993; Rickert et al. 1984). The DNA adducts formed after exposure to 2,4- or 2,6-DNT were persistent over time; the persistence of these adducts was slightly >40% in the 2 weeks after exposure (La and Froines 1992).

Comet assays, which evaluated the ability of DNT to induce DNA damage in the hepatocytes of treated rats, were negative for 2,3-, 2,4-, 2,5-, and 3,5-DNT (administered via gavage at up to 275, 142, 308, or

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39 mg/kg/day, respectively), but positive in Sprague-Dawley rats treated with 2,6-DNT via gavage at 35, 67, or 134 mg/kg/day (Lent et al. 2012; Suzuki et al. 2011).

Studies of the effects of various DNT isomers (2,4-, 2,6-, or 3,5-DNT) on sperm morphology (Soares and Lock 1980), spermatocyte DNA repair (Working and Butterworth 1984), and dominant lethal mutations (Soares and Lock 1980, U.S. Army 1979) were generally negative for these specific end points. Mice administered 2,4-DNT at 250 mg/kg on two consecutive days via gavage or intraperitoneal injection did not show evidence of an increase in morphologically aberrant sperm (Soares and Lock 1980), and UDS was not induced in the spermatocytes of rats treated with a single dose of 20 mg/kg 2,6-DNT via gavage (Working and Butterworth 1984). Dominant lethal effects were not induced in rats administered 2,4-DNT at up to 0.07% (34 mg/kg) in the diet for 13 weeks (U.S. Army 1979) or in mice administered 250 mg/kg 2,4-DNT or 3,5-DNT on two consecutive days via gavage or intraperitoneal injection (Soares and Lock 1980). However, a significant increase in the number of dead implants/total implants was reported in females mated to males treated with 2,4-DNT at 0.2% in the diet for 13 weeks (Hodgson et al. 1976).

3.4 TOXICOKINETICS

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

There are no available studies on absorption of inhaled 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT in humans or laboratory animals. However, based on analyses of the urinary metabolites of workers in DNT manufacturing plants (Levine et al. 1985b; Turner 1986; Woollen et al. 1985), it is apparent that inhalation absorption of DNT appears to occur under occupational settings where exposure may be higher than normal.

3.4.1.2 Oral Exposure

Rickert et al. (1983) suggested that the rapid disappearance of radioactivity from the first quarter of the small intestine of rats following the oral administration of uniformly [^{14}C]-ring-labeled 2,4- or 2,6-DNT indicates rapid and fairly complete absorption.

Excretion data and observed systemic effects indicate that DNT is absorbed following oral administration to experimental animals. Several strains of rats, New Zealand rabbits, beagle dogs, and Rhesus monkeys

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excreted 55–90% of the radioactivity from orally-administered radiolabeled DNT in the urine, primarily within the first 24 hours (Long and Rickert 1982; Rickert and Long 1981; U.S. Army 1978b). In mice, most of the radioactivity from ^3H -labeled 2,6-DNT was excreted in the urine (about 50% in 8 hours) (Schut et al. 1983), whereas most of the radioactivity from ^{14}C -labeled 2,4-DNT administered to mice was excreted in the feces, and only about 10% was excreted in the urine (U.S. Army 1978b). Increased fecal excretion could be due to reduced absorption or to greater excretion via the bile.

Studies on absorption of 2,3-, 2,5-, 2,6-, or 3,5-DNT following oral exposure of humans or animals were not identified.

3.4.1.3 Dermal Exposure

There were no *in vivo* data available specifically on the absorption of 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT via the dermal route of exposure. Two studies of occupational exposure to Tg-DNT have suggested that dermal absorption can be a significant route of entry for DNT in humans since the levels of urinary metabolites of 2,4- and 2,6-DNT in loaders and operators at a DNT manufacturing plant exceeded those that would have resulted from the inhaled concentrations (Levine et al. 1985b; Woollen et al. 1985). An *in vitro* study examined the dermal absorption of 2,4- and 2,6-DNT in soil or acetone using excised pigskin (Reifenrath et al. (2002). When in acetone, 36 and 24% of the 2,4- and 2,6-DNT, respectively, was absorbed. The absorption of radiolabel from soil was less; 15 or 16%, respectively, was absorbed from a low carbon soil and 5.4 and 3.8%, respectively, was absorbed from a high carbon soil.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No studies were located regarding distribution in humans or animals following inhalation exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

3.4.2.2 Oral Exposure

The tissue distribution of 2,4-DNT and its metabolites was studied by Rickert and Long (1980). 2,4-DNT was administered orally to male and female rats at doses of 10, 35, or 100 mg of ^{14}C -labeled 2,4-DNT/kg. When distribution is studied solely by detecting a radioisotope label, it is the labeled atom(s) that are being followed, and this label may be part of either the parent DNT molecule or a metabolite. Peak

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concentrations of radioactivity in plasma, red blood cells, liver, and kidney were proportional to dose. Levels in liver and kidney were 5–10 times higher than those in plasma or red blood cells. Levels of radioactivity in other tissues were lower than those in plasma. The only clear differences between males and females were the higher retention of radioactivity in red blood cells of females and the concentration of radioactivity in livers of females, which was only half that found in males. In addition, concentrations of 2,4-DNT in male kidneys peaked at 4–8 hours and were 3–10 times higher than the concentrations in female kidneys, which peaked 1 hour after the dose.

Rickert et al. (1983) observed that hepatic concentrations of radioactivity in male rats increased in two stages, with the first peak occurring 1–2 hours and a second peak occurring 8–12 hours after an oral dose of 10 or 35 mg/kg of radiolabeled 2,4- or 2,6-DNT. The second peak was followed by a gradual decline up to 16 days and was thought to be the result of enterohepatic cycling.

In mice administered ³H-labeled 2,6-DNT, the distribution of the label was similar in the blood, liver, kidneys, lungs, and small and large intestines at 8 hours after administration, with very low levels detected in the brain, lungs, heart, and spleen (Schut et al. 1983).

In a radioisotope labeling study in dogs and monkeys, total 2,4-DNT and its metabolites recovered in blood and other tissues were approximately 3.6% (dogs) and 2.2% (monkeys) of the administered dose (U.S. Army 1978b). Relative to blood concentrations, the liver had the highest levels of 2,4-DNT or metabolites. Detectable levels of 2,4-DNT or metabolites were also found in the kidney and in skeletal muscle (U.S. Army 1975, 1978b).

No studies were located regarding distribution in humans or animals following inhalation exposure to 2,3-, 2,5-, or 3,5-DNT.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals following dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

3.4.3 Metabolism

The metabolism of DNT in humans has been studied in workers exposed to Tg-DNT by the analysis of urinary metabolites. The routes of exposure in these studies were multiple. Since the amounts of

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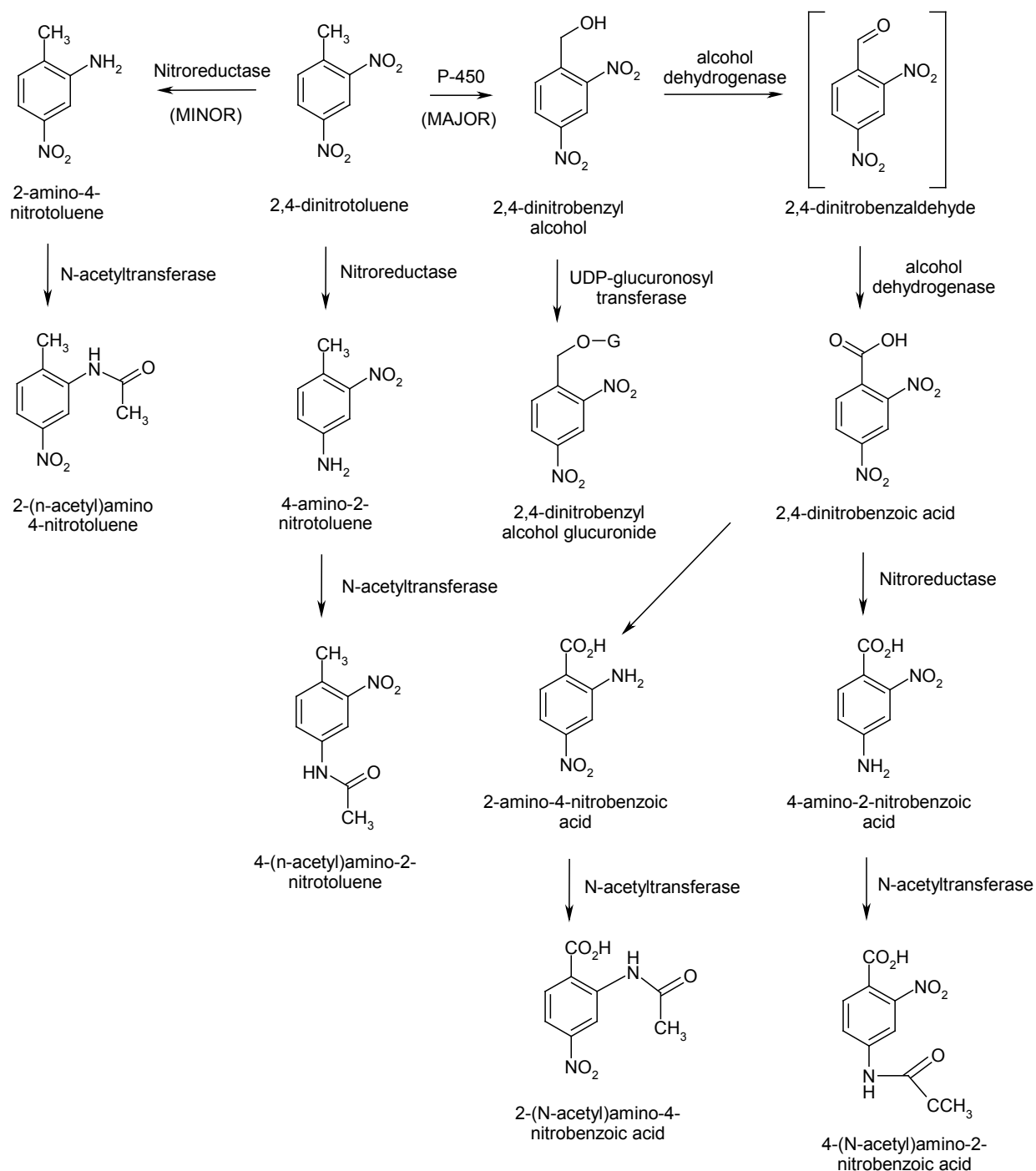
metabolites excreted could not be accounted for by the inhalation exposure route alone, dermal contact and ingestion routes of exposure may also be of importance (Levine et al. 1985b; Woollen et al. 1985). Woollen et al. (1985) found that the major metabolite excreted in the urine of workers exposed to Tg-DNT was 2,4-dinitrobenzoic acid (conjugates were hydrolyzed before analysis). There were wide variations in the excretion of the metabolites in different workers. Concentrations of 2,4-dinitrobenzoic acid in end-of-shift urine samples from 20 male and 8 female workers, however, did not suggest a difference in the excretion of this metabolite between males and females. The study authors stated that lesser amounts of the following metabolites were also found in the urine: 2-amino-4-nitro-, 4-amino-2-nitro-, and 2-amino-6-nitrobenzoic acids, and 4-(*N*-acetyl)amino-2-nitrobenzoic acid. Trace levels of DNT were also detected. Dinitrobenzyl alcohols were not detected. Neither amounts nor relative percentages of metabolites were reported.

Studies of workers at a Tg-DNT manufacturing plant (Levine et al. 1985b; Turner et al. 1985) provide more detailed information regarding the metabolism of Tg-DNT in occupationally exposed men and women. The principal metabolites detected in the urine of 14 men were dinitrobenzoic acids (2,4- and 2,6-) and 2-amino-4-nitrobenzoic acid. In the urine of three women, these metabolites were detected together with dinitrobenzyl alcohol glucuronides (2,4- and 2,6-). Expressed as percent of total urinary metabolites, the dinitrobenzoic acids, 2-amino-4-nitrobenzoic acid, and the dinitrobenzyl glucuronides constituted 52.5, 37.2, and 9.5%, respectively, of the total urinary DNT metabolites in men and 28.8, 37.6, and 33.3%, respectively, of the total urinary DNT metabolite in women. 2,4-DNT and 2,6-DNT metabolites were present in roughly the same proportions as in the Tg-DNT. Both men and women excreted relatively small amounts (<1% of urinary metabolites) of 2-(*N*-acetyl)amino-4-nitrobenzoic acid (Levine et al. 1985b).

In contrast to the findings in the Levine et al. (1985b) study, 2,4-dinitrobenzoic acid was not one of the primary urinary metabolites in Chinese workers exposed to Tg-DNT and mononitrotoluenes (Jones et al. 2005b). In these workers, 2,6-dinitrobenzoic acid, 4-amino-2-nitrobenzoic acid, 2-amino-4-nitrobenzoic acid, and 2,6-dinitrobenzyl alcohol comprised 27.3, 26.0, 21.6, and 18.3%, respectively, of the total DNT metabolites.

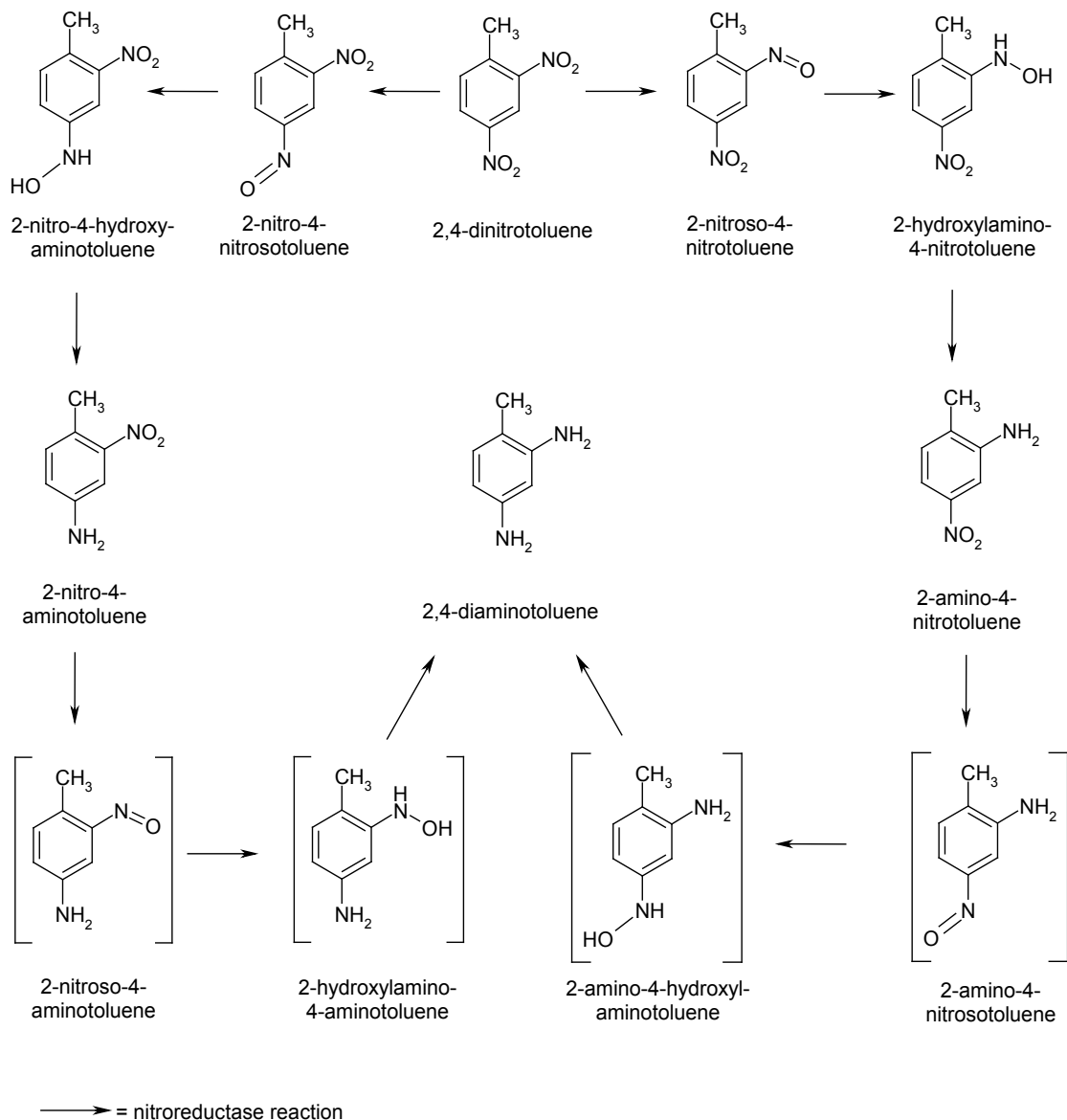
Studies in rats have identified a complex pathway for the metabolism of 2,4-DNT (Figures 3-3 and 3-4) and 2,6-DNT (Figure 3-5). Metabolism occurs in the liver and also in the intestine by microflora (Long and Rickert 1982; Rickert et al. 1981). Both oxidized and reduced metabolites are excreted in the urine after oral administration of the compounds. The main urinary metabolites of 2,4- and 2,6-DNT are the

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Figure 3-3. Proposed Metabolic Pathways for the Hepatic Metabolism of 2,4-DNT

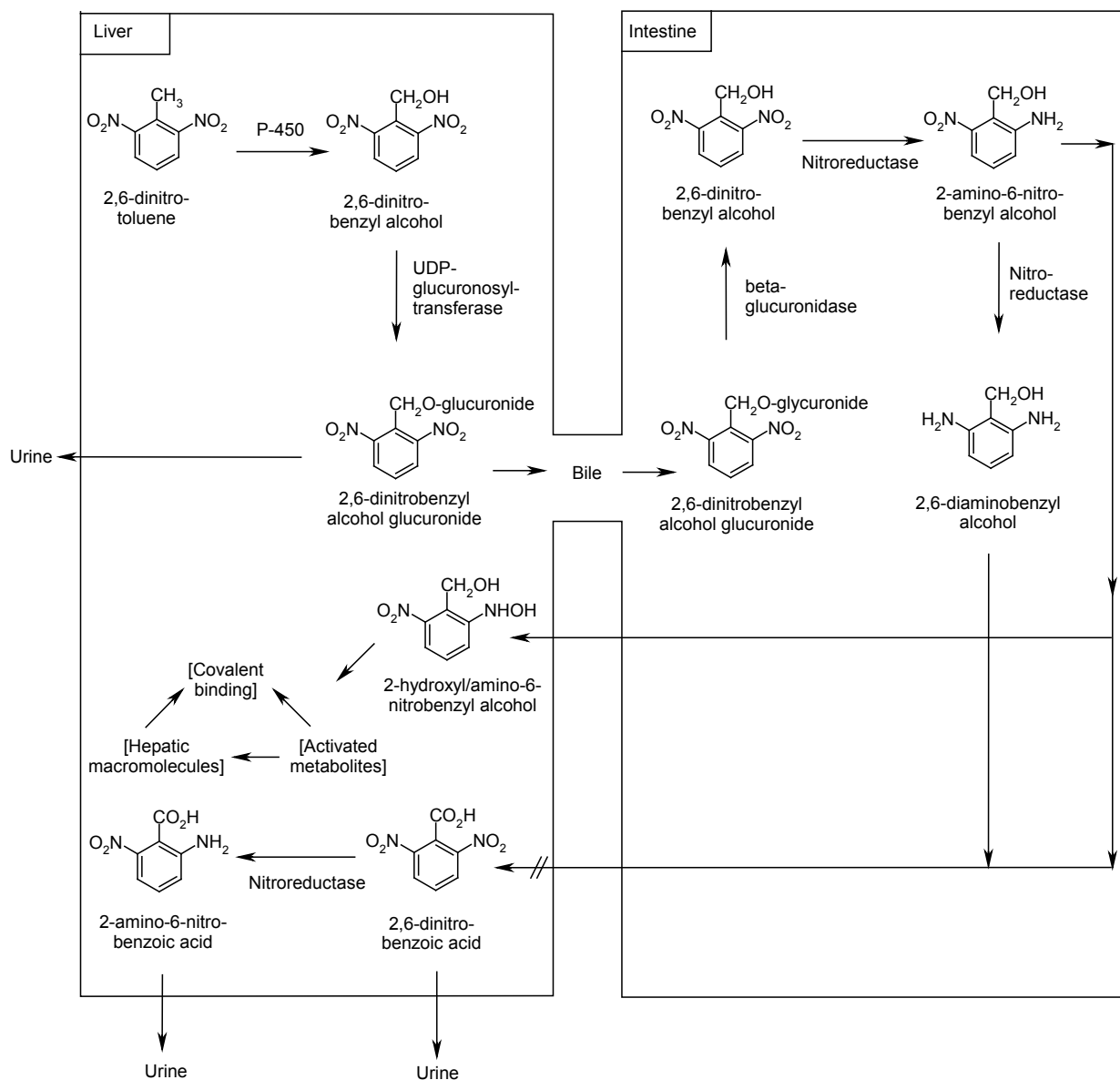
Sources: Bond and Rickert 1981; Bond et al. 1981; Smith et al. 1995

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Figure 3-4. Proposed Pathways for the Anaerobic of 2,4-DNT in Rat Intestinal Microflora

Sources: Guest et al. 1982; Mori et al. 1985

Figure 3-5. Proposed Pathways for Metabolism of 2,6-DNT



Sources: Chapman et al. 1993; La and Froines 1993; Rickert et al. 1984; Smith et al. 1995

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corresponding dinitrobenzyl alcohol glucuronide, dinitrobenzoic acid, and aminonitrobenzoic acid (Long and Rickert 1982). An additional urinary metabolite of 2,4-DNT is 4-(*N*-acetyl)amino-2-nitrobenzoic acid (Rickert et al. 1981). Although very little information on metabolism of 2,3-, 2,5-, or 3,5-DNT was identified, it is anticipated that metabolism of these isomers would follow similar pathways as those identified for 2,4- and 2,6-DNT. Results of an *in vitro* metabolism study using rat liver homogenate show that 2,3- and 2,5-DNT are reduced to monoaminonitrotoluenes and hydroxylaminonitrotoluenes (Kozuka et al. 1978).

Oxidative metabolism by cytochrome P450 predominates in the liver of experimental animals, leading to the formation of dinitrobenzyl alcohol, which is either converted to glucuronide conjugate or further oxidized to dinitrobenzoic acid. Dinitrobenzyl alcohol glucuronide is partially excreted into the bile, followed by metabolism by gut microflora and enterohepatic cycling (Long and Rickert 1982; Medinsky and Dent 1983; Mori et al. 1997; Rickert and Long 1981). Thus, DNT appears to be first metabolized by the liver with the metabolites being excreted into the bile; the biliary metabolites are hydrolyzed and further metabolized in the intestine; after reabsorption and circulation back to the liver, the metabolites are activated and bound to macromolecules (Chadwick et al. 1993; Long and Rickert 1982).

2,4- and 2,6-dinitrobenzyl glucuronide have been detected directly in the bile following administration of 2,4- and 2,6-DNT to the male Wistar rat (Mori et al. 1997), accounting for about 35 and 51% of the dose respectively. Four other metabolites, 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2,4-diaminotoluene, and 4-acetylamino-2-nitrobenzoic acid accounted for 0.02–0.12% of the dose; in addition to 2,4-dinitrobenzyl alcohol, 2,4-dinitrobenzaldehyde, and 2,4-dinitrobenzoic acid (0.09–0.14%) were detected in the bile of rats given 2,4-DNT. 2,6-Dinitrobenzyl alcohol, 2-amino-6-nitrotoluene, and 2,6-dinitrobenzaldehyde were detected in the bile of rats given 2,6-DNT.

Reductive metabolism of 2,3-, 2,4-, 2,5-, and 2,6 DNT occurs by *Escherichia coli* isolated from human intestine (Mori et al. 1984). For all isomers, *E. coli* reduced DNT to monoaminonitrotoluenes and hydroxylaminonitrotoluenes. Results are consistent with *E. coli* reduction of DNT via hydroxylaminonitrotoluenes to monoaminonitrotoluenes. Study authors suggest that reduced metabolites of DNT may play a role in the development of DNT-induced methemoglobinemia and anemia observed in humans and animals.

Studies of the metabolism of 2,4-DNT by intestinal microflora in rats and mice (Guest et al. 1982; Mori et al. 1985) and studies in germ-free rats (Rickert et al. 1981) have shown that intestinal microflora are

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responsible for reductive metabolism of DNT. Intestinal microorganisms hydrolyze and reduce 2,4- and 2,6-dinitrobenzyl alcohol glucuronide to the corresponding aminonitrotoluenes, probably through nitroso derivatives and hydroxylamino derivatives (Mori et al. 1997). The deconjugated metabolites are reabsorbed and transported back to the liver by enterohepatic circulation (Medinsky and Dent 1983). In the liver, the newly formed amine group is N-hydroxylated by cytochrome P450 and conjugated with sulfate (Kedderis et al. 1984). The sulfate conjugate is unstable and can be decomposed to form a carbonium or nitrenium ion that can be bound to hepatic macromolecules; this ostensibly leads to mutations and the formation of liver tumors. Thus, sulfation may be involved in the initiation stage of hepatocarcinogenesis by 2,6-DNT. Metabolism by intestinal microflora appears to be essential for the production of metabolites that bind covalently to liver macromolecules.

The intestinal biotransformation of 2,6-DNT was investigated *in vitro* using suspended microflora preparation from the intestinal contents of male Wistar rats (Sayama et al. 1993). It was determined that the metabolites formed with the incubation of 2,6-DNT were 2-nitroso-, 2-hydroxyl amino-, and 2-amino-6-nitrotoluene and 2,6-diaminotoluene. Since no metabolites were detected when 2,6-diaminotoluene was incubated and the recovery of 2,6-diaminotoluene was about 95%, it appears that 2,6-diaminotoluene is the terminal intestinal metabolite of 2,6-DNT (Sayama et al. 1993). When 2,4-DNT was examined in this system, two nitroazoxy compounds (2,2'-dimethyl-5-5'-dinitroazoxybenzene and 4,4'-dimethyl-3,3'-dinitro azoxybenzene) were detected in addition to other known metabolites, such as nitrosonitrotoluenes, hydroxyl aminonitrotoluenes, aminonitrotoluenes, and diaminotoluene (Sayama et al. 1993). The nitroazoxy compounds were believed to be non-enzymatic products (Sayama et al. 1993).

The metabolites formed by the anaerobic incubation of potassium 2,4-dinitrobenzyl glucuronide or potassium 2,6-dinitrobenzyl glucuronide with rat intestinal microflora have been examined (Mori et al. 1997). Metabolites transformed from 2,4-dinitrobenzyl glucuronide were 2,4-dinitrobenzyl alcohol, 4-amino-2-nitrobenzyl alcohol, and 2-amino-4-nitrobenzyl alcohol, which peaked at 30, 75, and 120 minutes of the incubation. 2,6-Dinitrobenzyl alcohol and 2-amino-6-nitrobenzyl alcohol were detected from potassium 2,6-dinitrobenzyl glucuronide incubation. Thus, intestinal metabolism includes the deconjugation of the glucuronide and the reduction of the nitro compound.

In rats, sex differences in the metabolism of 2,4-DNT have been observed. A larger percentage of the administered dose is excreted in the bile of male rats than is excreted in the bile of females. In females, a greater percentage of the dose is excreted in urine as the dinitrobenzyl alcohol glucuronide (Medinsky and Dent 1983; Rickert and Long 1981). The quantitative differences in urinary versus biliary excretion of

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the glucuronide conjugates by females may account for the sex differences in the susceptibility of the rat to the hepatocarcinogenic effects of 2,4-DNT (U.S. Army 1979). Greater urinary excretion may decrease the amount of the glucuronide available to the intestinal microflora for metabolism to a carcinogenic metabolite.

Metabolism studies in rats, rabbits, dogs, and monkeys with 2,4-DNT revealed the major urinary metabolites as glucuronide conjugates of 2,4-dinitrobenzyl alcohol (20–33% of the dose) and 2,4-aminonitro-benzyl alcohols (8–19% of the dose). Lesser amounts of aminonitrotoluene, 2,4-diaminotoluene, 2,4-aminobenzyl alcohol, and 2,4-dinitrobenzoic acid were also identified in all four species. Mice were also evaluated in this same group of studies. In mice, approximately 3% of the administered dose was excreted in the urine as the glucuronide conjugate of the 2,4-dinitrobenzyl alcohol and approximately 3% as the glucuronide conjugates of 2,4-aminonitrobenzyl alcohol (U.S. Army 1978b).

Another study using rats dosed with either 2,4- or 2,6-DNT also demonstrated that the primary urinary conjugate was the respective dinitrobenzyl alcohol glucuronide (11–17% of administered dose) (Mori et al. 1996). Other metabolites in rats administered 2,4-DNT included 2-amino-4-nitrobenzoic acid (0.71%), 4-amino-2-nitrobenzoic acid (0.52%), 4-acetylamino-2-nitrobenzoic acid (3.9%), 4-amino-2-nitrotoluene (0.04%), 2,4-dinitrobenzyl alcohol (0.25%), 2,4-dinitrobenzoic acid (6.9%), and 4-acetylamino-2-aminobenzoic acid (3.4%). After administration of 2,6-DNT, other metabolites in urine included 2,6-dinitrobenzoic acid (0.17%), 2-amino-6-nitrotoluene (0.44%), and 2,6-dinitrobenzyl alcohol (0.53%) (Mori et al. 1996).

The urinary metabolites of DNT, and probably the glucuronides resulting from occupational exposure of humans, are qualitatively the same as those resulting from oral administration to rats, but the proportions of nitro-reduced metabolites were lower relative to oxidized metabolites in the urine from humans (Turner et al. 1985). These differences may be due more to the particular routes of exposure (inhalation and dermal for humans; oral for rats) than differences in species. As seen in experimental animals, female subjects excreted a higher proportion of urinary metabolites as dinitrobenzyl alcohol glucuronides than did males.

Metabolism of DNT has not been studied in children. However, fetuses and neonates have been shown to be limited in their ability to biotransform xenobiotics. Although the cytochrome P450 isoforms responsible for DNT metabolism have not been identified, cytochromes CYP2E1, CYP2B1/2, and CYP2C11/6 are known to contribute to the side-chain oxidation of toluene by the rat liver, and multiple

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cytochrome P450 isoforms may contribute to the side-chain oxidation of DNT (Chapman et al. 1993). In humans, CYP2E1 protein is absent from fetal and neonatal livers, but steadily increases during the first year of life (Vieira et al. 1996). Other isoforms' expression in fetuses and neonates is also qualitatively and quantitatively different from the expression observed in adults (Komori et al. 1990; Leeder and Kearns 1997). In rats, while sulfotransferase (the enzyme that catalyzes sulfation) activity is almost at adult levels at birth, UDP-glucuronosyltransferase (the enzyme that produces glucuronide conjugates) activity towards different xenobiotics varies with maturation (Young and Lietman 1978). Similarly, in humans, sulfation capabilities develop faster than glucuronidation capabilities (Leeder and Kearns 1997). While the activity of some isoforms of sulfotransferase may exceed those seen in adults during infancy and early childhood, the activity of UDP-glucuronosyltransferase depends on the specific isoforms of the enzyme, and adult levels are generally attained by 6–18 months (Leeder and Kearns 1997). Since DNT undergoes bioactivation in the liver and by the intestinal microflora, the toxicity of DNT may be different in children. Newborns have a transient deficiency in methemoglobin reductase (Gruener 1976) and have a high concentration of fetal hemoglobin in their erythrocytes. Consequently, they are highly sensitive to methemoglobin-generating chemicals and to methemoglobinemia generated by DNT.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

In occupational settings, in addition to inhalation, some oral and dermal exposure can occur. The elimination of DNT in the urine of workers exposed to Tg-DNT has been studied by several investigators (Levine et al. 1985b; Turner et al. 1985; Woollen et al. 1985).

Woollen et al. (1985) observed that the highest rates of excretion of 2,4-dinitrobenzoic acid occurred near the end of the work shift. The half-life for urinary excretion of 2,4-dinitrobenzoic acid was calculated to be 2–5 hours. This estimate appears to be the initial phase of a biphasic elimination profile since even 3 days after the last exposure, detectable levels of 2,4-dinitrobenzoic acid were present in urine.

Turner et al. (1985) determined the metabolic profiles in workers exposed to DNT. The half-life for excretion of DNT metabolites in urine ranged from 0.8 to 4.5 hours. The half-lives for 2,4-dinitrobenzoic acid and 2,4-dinitrobenzyl alcohol glucuronide tended to be shorter than those for the metabolites that resulted from both oxidative and reductive metabolism.

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No studies investigating elimination and excretion of 2,3-, 2,5-, 2,6-, or 3,5-DNT following inhalation exposure of humans or animals were located.

3.4.4.2 Oral Exposure

No studies were located regarding excretion in humans following oral exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

Schut et al. (1983) reported that in mice, urine was the main route of elimination of ³H-labeled 2,6-DNT, with about 50% excreted after 8 hours. U.S. Army (1978b) observed that most of the radioactivity from ¹⁴C-labeled 2,4-DNT administered to mice was excreted in the feces and only about 10% in the urine. Differences between these two studies could be due, in part, to the use of different species of mice.

Male and female rats excreted 55–90% of the radioactivity from ¹⁴C-2,4-DNT or ¹⁴C-2,6-DNT in the urine, and 15–30% in the feces, within 72 hours after dosing (Long and Rickert 1982; Rickert and Long 1981). With 2,4-DNT, the females excreted a greater percentage of the dose in the urine as 2,4-dinitrobenzylalcohol glucuronide than did the males (except at the highest dose), but with 2,6-DNT, no sex-related difference in urinary excretion was seen.

In experiments with bile duct-cannulated rats, male rats excreted 25% of the radioactivity from ¹⁴C-2,4-DNT into the bile over a 36-hour period, whereas female rats excreted 18% (Medinsky and Dent 1983). Biliary excretion of radioactivity was linearly related to dose in males; females were evaluated only at one dose. Biliary excretion of radioactivity was virtually complete within 24 hours for males and 12 hours for females. Mean half-times of biliary excretion ranged from 3.3 to 5.3 hours. Urinary excretion was also significant, with greater amounts of radioactivity excreted in the urine of rats from which bile was not collected (60–90% of the dose) than in the urine of rats from which bile was collected (20–60% of the dose). This finding indicates that biliary metabolites were absorbed from the intestines (enterohepatic cycling). Whether or not bile was collected, female rats excreted more radioactivity in urine than did male rats. Greater than 90% of the urinary excretion of labeled metabolites appeared in urine collected during the first 24 hours. At the end of 36 hours, only 0.02–0.05% of the radioactivity was detectable in the livers; 20–60% of this was covalently bound.

No studies investigating elimination and excretion of 2,3-, 2,5-, or 3,5-DNT following oral exposure of humans or animals were located.

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3.4.4.3 Dermal Exposure

There are no kinetic data in humans in which the route of exposure was specifically dermal. Occupational exposure studies available for Tg-DNT involved multiple routes of exposure (Levine et al. 1985b; Turner et al. 1985; Woollen et al. 1985). The major routes of exposure in these studies were considered to be inhalation and dermal. The results were discussed previously in Section 3.4.4.1.

No studies were located regarding excretion in animals following dermal exposure to 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

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

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

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

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen

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1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

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

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

Figure 3-6 shows a conceptualized representation of a PBPK model.

If PBPK models for 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

A PBPK model has not been developed for 2,3-, 2,4-, 2,5-, 2,6-, or 3,5-DNT.

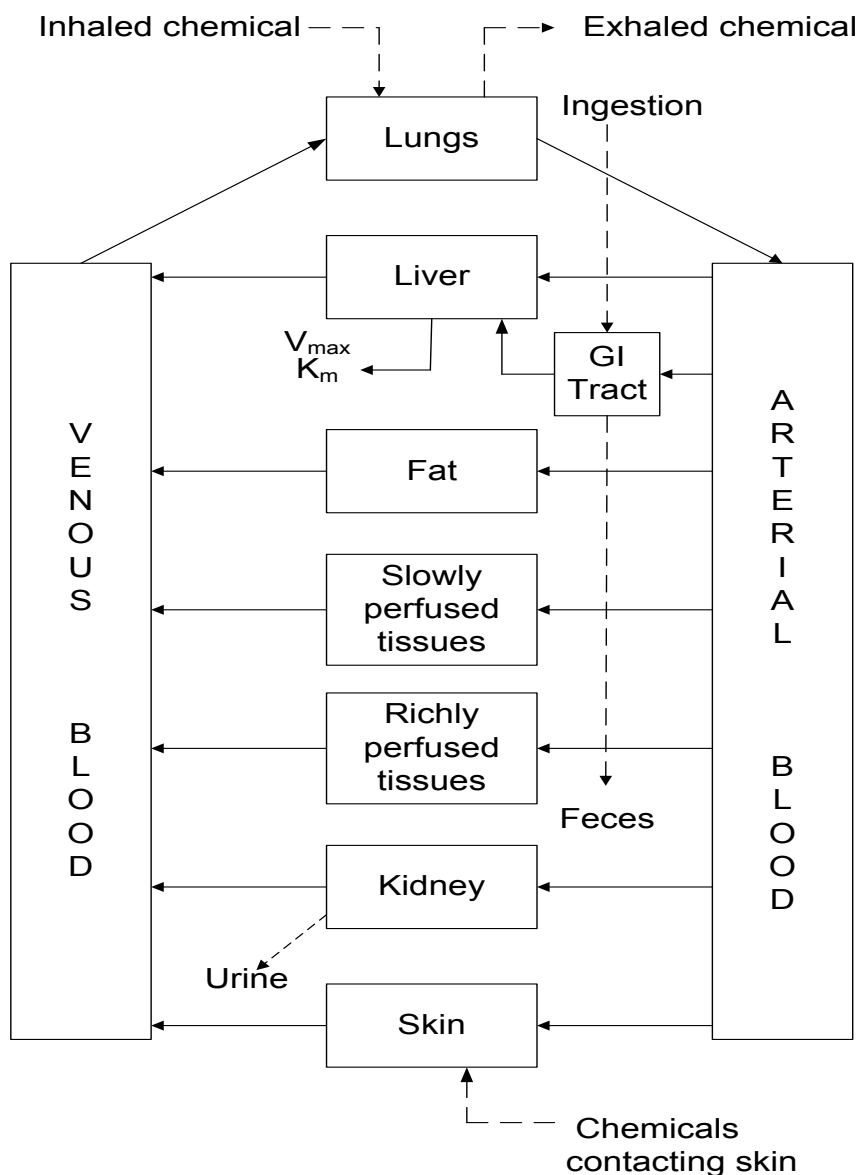
3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

No information was located regarding the mechanism of absorption of 2,4- or 2,6-DNT. It is known that absorption occurs after inhalation exposure based on the metabolites found in the urine of workers at DNT manufacturing plants (Levine et al. 1985b; Turner 1986; Woollen et al. 1985). In studies of rats, rabbits, dogs, and monkeys, most orally administered 2,4- or 2,6-DNT has been shown to be absorbed

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Figure 3-6. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



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

Source: adapted from Krishnan and Andersen 1994

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(Long and Rickert 1982; Rickert and Long 1981; U.S. Army 1978b). There appears to be minimal accumulation of these compounds after a single exposure. After repeated oral exposure in rats, 2,4-DNT and its metabolites were preferentially distributed to the liver, kidney, brain, lung, and skeletal muscle. The primary metabolite of 2,4-DNT excreted by humans exposed via inhalation and dermal routes of exposure in occupational studies or animals exposed via the oral route is 2,4-dinitrobenzyl alcohol and/or its glucuronide (EPA 1992). In addition to this, humans also excrete 2-amino-4-nitrobenzyl alcohol in the urine. 2,4-Dinitrobenzoic acid is another major metabolite (EPA 1992). Both 2-nitroso-4-nitrotoluene and 2-amino-4-nitrotoluene, metabolites of 2,4-DNT in humans, have been shown to be mutagenic *in vitro* (EPA 1992). It has been suggested that these intermediates may bind covalently to hepatic macromolecules, such as DNA and RNA (EPA 1992).

3.5.2 Mechanisms of Toxicity

Effects of Metabolism on Toxicity. The primary mechanism of toxicity for DNT involves bioactivation to form reactive intermediates (Kedderis et al. 1984; Sayama et al. 1989b). Detailed information on the biotransformation of DNT is presented in Section 3.4.3. Briefly, metabolism of DNT begins in the liver, where it is oxidized by cytochrome P450 and conjugated with glucuronic acid to form the major metabolite, dinitrobenzyl alcohol glucuronide, and is excreted in bile or urine (Long and Rickert 1982; Medinsky and Dent 1983). The glucuronide excreted in bile undergoes biotransformation by intestinal microflora, where the conjugate is hydrolyzed and subsequently reduced by nitroreductase to the corresponding aminonitrobenzyl alcohol (Chadwick et al. 1993; Guest et al. 1982; Mori et al. 1985), probably through nitroso derivatives and hydroxylamino derivatives. The deconjugated metabolites are reabsorbed and transported back to the liver by enterohepatic circulation (Medinsky and Dent 1983). In the liver, the newly formed amine group is N-hydroxylated by cytochrome P450 and conjugated with sulfate (Kedderis et al. 1984). The sulfate conjugate is unstable and can be decomposed to form a carbonium or nitrenium ion that can be bound to hepatic macromolecules; this ostensibly leads to mutations and the formation of liver tumors. Thus, sulfation may be involved in the initiation stage of hepatocarcinogenesis by 2,6-DNT.

Target Organ Toxicity. The mechanism of toxicity of the hematological effects of DNT is described by U.S. Army (1979). The effect of DNT on the blood is also produced by aromatic amines and most organic and inorganic nitrates. These compounds or their metabolites oxidize the ferrous ion in hemoglobin and produce methemoglobin. Hydroxylamine is probably the oxidizing species, because it is an intermediate in the reduction of nitro to amines. Within limits, the body can correct

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methemoglobinemia, but the corrective measures can be overwhelmed, producing numerous secondary effects including anoxia. The presence of methemoglobin leads to the formation of aggregates of hemoglobin degradation products called Heinz bodies. The presence of Heinz bodies is a sensitive indicator of blood toxicity as it indicates that some hemoglobin has been destroyed. High levels of methemoglobin are removed by catabolism, leading to the development of anemia. The body compensates for the destruction of red blood cells by increasing erythrocyte production, resulting in large numbers of immature erythrocytes, called reticulocytes, in the blood. If the toxic dose is not too severe, these compensatory mechanisms suffice. Thus, "compensated anemia," normal erythrocyte levels with reticulocytosis, may exist in exposed individuals. When the production of red blood cells can no longer keep pace with the hemolysis, frank anemia may be present (U.S. Army 1979).

As discussed in Section 3.2.2.7 (Oral Exposure; Cancer), 2,4-, 2,6-, or Tg-DNT have been shown to induce hepatocellular carcinoma following chronic-duration oral exposure. Thus, hepatic effects observed at less than chronic exposure durations or at lower doses may represent early stages of progressive development to hepatic cancer or threshold level effects for development of cancer; however, the mechanism of toxicity for hepatic effects has not been elucidated. A study examining changes in gene expression in the livers of female rats administered 2,4-DNT at 5–398 mg/kg/day or 2,6-DNT at 5–199 mg/kg/day via gavage as a single dose showed that treatment with these compounds perturbed pathways involved in DNA damage response, cell death signaling, detoxification and lipid metabolism, the oxidative stress response, and the immune response 24 and 48 hours after dosing (Deng et al. 2011).

The mechanisms of toxicity for reproductive effects (testicular atrophy, degeneration of the seminal vesicles, and decreased sperm production) observed in animal studies (Bloch et al. 1988; U.S. Army 1976, 1978b, 1979) have not been elucidated. However, data from *in vitro* studies indicate that testicular degeneration may be associated with structural changes in Sertoli cells (Reader and Foster 1990). Bloch et al. (1988) reported that diminished sperm counts in rats acutely exposed to 2,4-DNT were accompanied by fine structural alterations of Sertoli cells. Increased serum levels of FSH, also indicative of Sertoli cell misfunction, were also reported (Bloch et al. 1988). Reader and Foster (1990) reported that Sertoli cell cultures prepared from the testes of Wistar rats and treated with up to 100 μ M 2,4- or 2,6-DNT remained intact; however, cells treated at 50 μ M of 2,4- or 2,6-DNT showed some evidence of damage (absence of germ cells and some Sertoli cells containing cytoplasmic vacuoles). Germ cell detachment from Sertoli germ cell cocultures was significantly increased ($p < 0.05$) compared to controls at 10 μ M 2,4- or 2,6-DNT (Reader and Foster 1990). Further evidence of disruption of Sertoli cell function was observed as increased production of lactate and pyruvate, although increases in pyruvate production were minimal

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with 2,6-DNT (Reader and Foster 1990). Another study showed that adult immortalized rat Sertoli cells exposed to 2,4-DNT at 25–200 $\mu\text{mol/L}$ showed increased numbers of autophagosomes and autophagic lysosomes, which may signal the removal of cellular components damaged by exposure or impending apoptosis (Sorenson and Brabec 2003). Thus, it appears that structural changes in the Sertoli cells may be precipitating events responsible for reproductive effects observed in animals exposed to DNT.

Carcinogenesis. The mechanisms of DNT-induced carcinogenicity have not been described. However, genotoxicity assays *in vitro* and *in vivo* indicate that DNT isomers have the potential to induce gene mutations and other forms of DNA damage (DNA adduct formation, chromosomal aberrations, and UDS). The mechanism underlying the development of renal tumors in CD-1 male mice exposed to oral 2,4-DNT at 95 mg/kg/day for 2 years (U.S. Army 1979) has not been determined. However, workers exposed occupationally to explosives containing 30% Tg-DNT with renal cancer typically showed pathological patterns of urinary excretion, indicated by the molecular weight of excreted proteins (i.e., increased excretion of tubular proteins of low molecular weight and glomerular leakage with excretion of high molecular weight proteins), and/or specific biomarkers of damage to the proximal tubule of the nephron (α_1 -microglobulin and glutathione-S-transferase α). These data are consistent with the hypothesis that renal tumors are initiated by DNT isomers and that promotion occurs via damage to the proximal tubule of the nephron (Brüning et al. 2001).

In hepatic tumor initiation-promotion experiments, Tg-DNT and 2,6-DNT were found to have tumor-promoting and tumor-initiating activity; 2,4-DNT showed only tumor-promoting activity (Leonard et al. 1983, 1986; Mirsalis and Butterworth 1982; Popp and Leonard 1982). 2,6-DNT was indicated to be a complete hepatocarcinogen and is primarily responsible for the carcinogenic activity of Tg-DNT. Hepatic DNA adducts have been detected by ^{32}P -postlabeling technique in 2,6-DNT-treated B6C3F₁ mice and F344 rats (George et al. 1996). In male Wistar rats administered 2,4- or 2,6-dinitrobenzyl glucuronide, the major compounds excreted in the bile after treatment with 2,4- or 2,6-DNT, secondary metabolism led to the production of genotoxic agents in the urine and bile (Mori et al. 2000). 2,4-Dinitrobenzaldehyde (mutagenic in the Ames assay using *Salmonella typhimurium* strains TA98 and TA100) and 2,4-diaminotoluene (carcinogenic) were detected in the urine and bile of 2,4-dinitrobenzyl glucuronide-treated rats; 2-amino-6-nitrobenzyl alcohol (genotoxic) was detected in 2,6-dinitrobenzyl glucuronide-treated rats. The formation of these metabolites may contribute to the hepatocarcinogenesis induced by 2,4- and 2,6-DNT. 2,6-Dinitrobenzaldehyde, another metabolite of 2,6-DNT, was found to be a direct-acting mutagen in the *Salmonella typhimurium* strain TA98 and TA100 systems, not requiring metabolic activation by the S9 mix. 4-Amino-2-nitrobenzyl alcohol, 2-amino 4 nitrobenzyl alcohol, and 2-amino-

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6-nitrobenzyl alcohol are also mutagenic metabolites of 2,4- and 2,6-DNT, with their mutagenicity requiring metabolic activation (Mori et al. 1982; Sayama et al. 1989b). Kedderis et al. (1984) proposed a bioactivation mechanism relating to the genotoxicity of 2,6-DNT in male F344 rats. They showed that the active metabolite of 2,6-DNT in the male F344 rat is the hydroxylamino sulfate of aminonitrobenzyl alcohol formed by the intestinal metabolism of benzyl glucuronide of 2,6-dinitrobenzyl alcohol excreted in bile. The sulfate conjugate is unstable, and the formation of electrophilic carbonium or nitrenium ions from these conjugates leads to subsequent binding to DNA.

3.5.3 Animal-to-Human Extrapolations

Correlation of toxic effects between humans and animals for 2,4- and 2,6-DNT with regard to hematologic and neurological effects has been noted (Ellis et al. 1985; Hong et al. 1985; Lane et al. 1985; Lee et al. 1985; McGee et al. 1947; U.S. Army 1978b, 1979). Other effects for 2,4- and 2,6-DNT, such as reproductive, hepatic, renal, and cancer have been noted in animals (Ellis et al. 1985; Hong et al. 1985; Lee et al. 1985; Leonard et al. 1983, 1986; McGown et al. 1983; Stoner et al. 1984; U.S. Army 1976, 1978b, 1979), but insufficient data are available to state definitively whether they are effects in humans. Two mutagenic metabolites of 2,4-DNT have been found in humans, mice, and rats (EPA 1992). Although rats appear to be more sensitive to the effects of 2,4- and 2,6-DNT than mice (Hong et al. 1985; Lane et al. 1985; U.S. Army 1975, 1978a, 1978b; Vernot et al. 1977), dogs appear to be the most sensitive of the three species (Ellis et al. 1985; U.S. Army 1976, 1978b, 1979). However, limited intermediate-duration data using 2,6-DNT have shown mice to be more sensitive than rats (U.S. Army 1976). It should be noted that dogs were fed DNT by capsule in experimental studies, whereas the rodents received the test chemical in feed (Ellis et al. 1985; Hong et al. 1985; Lee et al. 1985; U.S. Army 1975, 1976, 1978b, 1979).

Extrapolating animal toxicity data to predict human risk from exposure to 2,4- and 2,6-DNT appears to be reasonable because of qualitative similarities in metabolism and known toxic effects.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to

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develop a screening program for “...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...”. To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavonoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No studies were located regarding endocrine disruption in humans and/or animals after exposure to 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT.

No *in vitro* studies were located regarding endocrine disruption of 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT.

3.7 CHILDREN'S SUSCEPTIBILITY

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

Relevant animal and *in vitro* models are also discussed.

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Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The fetus/infant has an immature (developing) blood-brain barrier that past literature has often described as being leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-CNS interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the blood-brain barrier, there are differences between fetuses/infants and adults which are toxicologically important. These differences mainly involve variations in physiological transport systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential

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selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; as it raises a very important toxicological question as to whether these mechanisms provide protection for the developing brain or do they render it more vulnerable to toxic injury. Each case of chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

No specific health effects resulting from DNT exposure have been observed in children. Generally, health effects observed in adults should also be of potential concern in children.

No direct information is available regarding the effects of DNT on the developmental process in humans, and there are few developmental studies on animals. When Tg-DNT was administered by gavage to pregnant rats for 14 days during gestation, and pups were evaluated for developmental toxicity either at gestation day 20 or postpartum day 60 (Jones-Price et al. 1982), adverse effects on hematologic

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parameters and altered organ weights were observed in both dams and fetuses when dams were administered 100 or 150 mg/kg/day. However, the fetal toxicity was not dose-related. A decrease in relative liver weight was observed in the postpartum pups at the low dose of 14 mg/kg/day. Dose-related effects on postnatal development were not observed in pups when dams were administered 35 or 75 mg/kg/day.

No consistent changes were observed in the number of preimplantation losses, implantation sites, or living or non-living fetuses in male Sprague-Dawley rats gavaged with 2,4 DNT at 0, 60, 180, or 240 mg/kg/day for 5 days (Lane et al. 1985). Exposure of male animals to DNT does not cause dominant lethal mutation or increases in the proportion of nonviable conception (U.S. Army 1979).

DNT has been found to be genotoxic using *in vivo* test systems (Ashby et al. 1985; Huang et al. 1995; Mirsalis et al. 1989). Although 2,6-DNT itself showed no mutagenicity towards *Salmonella typhimurium* strains TA98 and TA100 with or without activation by S9 mix, 2,6-dinitrobenzaldehyde, a metabolite of 2,6-DNT, was found to be a direct-acting mutagen, not requiring metabolic activation (Sayama et al. 1989b). The reason that DNT is not shown to bind to DNA and cause mutations in most of the short-term *in vitro* assays for genotoxicity is that the formation of DNA-reactive DNT metabolites involved several different biotransforming enzymes in the intestinal microflora and in the liver. However, DNT did not cause dominant lethal mutation or increases in the proportion of nonviable conceptions following exposure of male animals (U.S. Army 1979), so it is not clear if the genotoxic form of DNT might potentially reach the germ cells following oral, inhalation, or dermal exposure.

It is unlikely that DNT and its metabolites will accumulate in maternal tissues because of its low octanol-water partition coefficient. No studies are available that demonstrate DNT or its metabolites cross the placenta or get into breast milk. Thus, it is unlikely that the developing fetus or nursing infant would be exposed to DNT as a consequence of maternal exposure prior to gestation. However, developmental toxicity from DNT could potentially occur because of its ability to deplete the amount of oxygen available to the developing fetus. Pregnant women and their fetuses may be susceptible to the oxygen depletion implied by the hematoxicity of DNT based on a study of rats (Jones-Price et al. 1982). Newborns have a transient deficiency in methemoglobin reductase which reduces methemoglobin back to hemoglobin (Gruener 1976). They also have a high concentration of fetal hemoglobin in their erythrocytes (Smith 1996). Thus, newborns are unusually sensitive to methemoglobin-generating chemicals such as DNT. The metabolism of DNT has not been studied in children or appropriate animal models. However, while some of the enzymes involved in DNT metabolism reach or exceed adult levels during infancy and early

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childhood, other enzymes such as UDP-glucuronosyltransferase may attain adult levels by 6–18 months of age (Leeder and Kearns 1997). Thus, the toxicity of DNT may be different in children.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

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, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-DNT are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by DNT are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or

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other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

There are no biomarkers of exposure effects that have been validated in children or in adults exposed as children.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to DNT

Workers exposed to DNT in a manufacturing plant excreted 2,4-DNT, 2,6-DNT, and their metabolites in the urine (Levine et al. 1985b). The concentrations of DNT in air ranged from 0.1 to 5.9 mg/m³. Concentrations of DNT and metabolites ranged from 1.68 to 16.74 mg/day (or 1.74–17.31 mg/L, based on an average daily urine volume of 967 mL), with widespread daily variations. Estimates of inhaled DNT ranged from 0.5 to 4.9 mg/day, less than the total excreted suggesting that dermal exposure, and possibly oral exposure, contributed to the body burden of DNT. Jones et al. (2005b) did not find a significant correlation between the levels of air concentrations of 2,4- and 2,6-DNT and the sum of urine metabolites among workers at a DNT and mononitrotoluene manufacturing facility. The 8-hour TWA air concentrations were 0.043 and 0.014 mg/m³ for 2,4- and 2,6-DNT, respectively.

Woollen et al. (1985) determined that the urinary concentration of a Tg-DNT metabolite, 2,4-dinitrobenzoic acid, was <1 mg/L at the beginning of the work week and ranged from 3.4 to 41 mg/L at the end of the shift. Atmospheric DNT levels of undetectable to 0.03 mg/m³ were monitored with personal air samples. Static samples near dusty process areas monitored were 0.02–2.68 mg/m³. The study authors estimate that inhalation exposures ranged from 1 to 14 mg/day. As in Levine et al. (1985b), inhalation exposure does not account for the entire amount of DNT metabolites excreted in urine. Dermal and ingestion exposures are, therefore, likely to have occurred.

Significant correlations between urinary levels of hemoglobin adducts and urinary metabolites were found for 2,4- and 2,6-DNT in workers exposed to Tg-DNT (Sabbioni et al. 2006). The hemoglobin adducts resulting in exposure to 2,4-DNT were 4-amino-2-nitrotoluene and 2,4-toluenediamine; the hemoglobin adducts from 2,6-DNT exposure were 2-amino-6-nitrotoluene and 2,6-toluenediamine. The best correlations between urinary metabolites and hemoglobin adducts were 2,4-dinitrobenzyl alcohol and 2,6-dinitrobenzyl alcohol with hemoglobin 2,4-nitrotoluene and hemoglobin-2-amino-6-nitrotoluene, respectively.

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3.8.2 Biomarkers Used to Characterize Effects Caused by DNT

For more information on biomarkers for renal and hepatic effects of chemicals see Agency for Toxic Substances and Disease Registry/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990).

After exposure to DNT, methemoglobin levels in the blood may be elevated (Ellenhorn 1997). The methemoglobinemia present may be quite profound and its onset is often delayed by up to 4 hours (Ellenhorn 1997). Another hematological change that might be present in individuals who have undergone repeated or prolonged exposure to DNT is that which is consistent with Heinz bodies and hemolytic anemia.

DNA adducts have been found in the livers of rats treated orally with either 2,4- or 2,6-DNT (La and Froines 1992, 1993). The formation of DNA adducts is believed to be indicative of carcinogenic risk.

Decreased spermatogenesis has been reported in treated rats, mice, and dogs (Bloch et al. 1988; U.S. Army 1979). However, decrease in sperm counts in workers exposed to DNT has been reported in only one study (CDC 1981).

3.9 INTERACTIONS WITH OTHER CHEMICALS

Perkins (1919) reported that “alcoholic subjects have very little resistance to DNT.”

Exposure of male rats to 2,6-DNT for 5 days reduced the rate of metabolism of phenobarbital; exposure to 2,6-DNT for 4 weeks increased phenobarbital metabolism (Short and Lee 1980). Exposure of rats to 2,4-DNT did not affect the rate of phenobarbital metabolism.

The effects of the herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), on 2,6-DNT genotoxicity were examined in male weanling F344 rats (George et al. 1992). The rats were treated orally with 54.4 mg/kg 2,4,5-T for 4 weeks, then with 75 mg/kg 2,6-DNT, 1, 2, or 4 weeks after the first dose of 2,4,5-T; urine was then collected for 24 hours. In animals treated for 1 week with 2,4,5-T, there was a decrease in transformation of 2,6-DNT to mutagenic metabolites in the urine, but there were no changes in intestinal enzyme activities (George et al. 1992). Longer treatments with 2,4,5-T did not alter urine genotoxicity

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compared to controls, and there was a transient increase in cecal azo reductase and nitroreductase after 2 weeks with a decrease in intestinal β -glucuronidase activity, but all levels were normal after 4 weeks.

Pretreatment of rats with alachlor for 3 weeks prior to a single oral administration of 75 mg/kg 2,6-DNT resulted in the production of genotoxic metabolites, as evaluated in a *S. typhimurium* histidine reversion bioassay in the absence of S9 activation (George et al. 1998). Significant increases, compared to 2,6-DNT-only exposed rats, were not observed in rats exposed to alachlor for 1 or 5 weeks.

Interaction of DNT with other chemicals has not been observed in children.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to 2,4- and 2,6-DNT than will most persons exposed to the same level of 2,4- and 2,6-DNT in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of 2,4- and 2,6-DNT, or compromised function of organs affected by 2,4- and 2,6-DNT. Populations who are at greater risk due to their unusually high exposure to 2,4- and 2,6-DNT are discussed in Section 6.7, Populations with Potentially High Exposures.

Several studies have examined the possible association between DNT toxicity and genetic polymorphisms. Sabbioni et al. (2006) found that workers exposed to DNT and TNT with the glutathione S-transferase T1 null genotype or *N*-acetyltransferase 1 fast acetylators genotype had significantly more chromatid-type chromosomal aberrations than controls of the same genotypes. Workers with the sulfotransferase 1A1 Arg/Arg or sulfotransferase 1A2 Asn/Asn genotypes or *N*-acetyltransferase 1 fast acetylators had significantly more total aberrations than controls with the same genotypes. These results suggest differences in susceptibility to DNT toxicity. In contrast, Brüning et al. (1999) found that all of the workers at a copper mine using Tg-DNT explosives with urothelial cancer were slow acetylators.

Humans sensitive to DNT may include individuals with cardiovascular problems. Hematological effects associated with exposure to 2,4-DNT may place persons with anemia, including sickle cell anemia or other diseases of the blood, at an increased risk.

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Persons with chronic neurological disorders may also have an increased sensitivity to DNT exposure. Although there are insufficient data available to draw firm conclusions, it appears that pregnant women and their fetuses may be susceptible to the oxygen depletion implied by the hematotoxicity of DNT based on a study on rats (Jones-Price et al. 1982). Although it has been reported that alcoholics may have a decreased resistance to the effects of Tg-DNT (Perkins 1919), the extent of this compromise has not been determined.

The susceptibility of children to the health effects of DNT may be different from that of adults, as discussed in Section 3.7.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 2,4- and 2,6-DNT. 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- and 2,6-DNT. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to 2,4- and 2,6-DNT:

Currance PL, Clements B, Bronstein AC. 2007. Dinitrophenol and related compounds. In: Emergency care for hazardous materials exposure. St. Louis, MO: Mosby Jems, 335-338.

Ellenhorn MJ. 1997. Ellenhorn's medical toxicology: Diagnosis and treatment of human poisoning, 2nd ed. Baltimore, MD: Williams and Wilkins, 1366-1368.

There are no known pediatric-specific methods for reducing peak absorption following exposure, reducing burden, or interfering with the mechanism of action for toxic effects.

3.11.1 Reducing Peak Absorption Following Exposure

Limited information from humans indicates that DNT is absorbed after inhalation exposure, while animal data suggest that DNT is rapidly and completely absorbed after oral exposure. Efforts to reduce absorption following acute exposure to DNT should focus on removing the individual from the site of exposure and decontaminating exposed areas of the body. Contaminated clothing and jewelry should be removed and skin should be washed with soap and water (Bronstein and Currance 1994). It is suggested that eyes exposed to DNT be copiously irrigated with water and normal saline (Bronstein and Currance

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1994). If ingestion of DNT occurs, it is suggested that the mouth be rinsed, and water can be administered for dilution if the patient can swallow, has a good gag reflex, and is not drooling (Bronstein and Currance 1994). In addition, the use of activated charcoal has been suggested (Bronstein and Currance 1994). Induction of emesis is contraindicated (Bronstein and Currance 1994). In patients who present within 2–4 hours of DNT ingestion, gastric lavage may be helpful in decreasing peak absorption following exposure (Ellenhorn 1997). There may also be some benefit in administering activated charcoal and cathartics after lavage (Ellenhorn 1997).

3.11.2 Reducing Body Burden

There are no data to support the use of hemodialysis, forced diuresis, hyperbaric oxygen, or hemoperfusion for treatment of methemoglobinemia alone, but these treatments may provide adjunctive care after DNT ingestion when supportive care is inadequate (Ellenhorn 1997).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Exposure to DNT can cause profound methemoglobinemia with its sequelae (cyanosis and Heinz body formation), anoxia, and death (Ellenhorn 1997). The antidote used for serious methemoglobinemia is methylene blue (tetramethylthionine chloride), but treatment with methylene blue is not indicated for all patients (Ellenhorn 1997). Treatment with methylene blue is believed to be effective because it acts as a cofactor to increase the erythrocyte reduction of methemoglobin in the presence of NADPH (Ellenhorn 1997). Methylene blue is oxidized and the resulting molecule becomes an electron donor for the nonenzymatic reduction of methemoglobin to oxyhemoglobin (Ellenhorn 1997). Exchange transfusion and/or packed red blood cell transfusion may be useful for patients who do not respond to methylene blue or for patients with G6PD- or NADPH-methemoglobin reductase deficiencies (Ellenhorn 1997).

3.12 ADEQUACY OF THE DATABASE

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

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

3.12.1 Existing Information on Health Effects of DNT

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 2,4-, 2,6-, and Tg-DNT are summarized in [Figures 3-7, 3-8, and 3-9](#), respectively. The purpose of these figures is to illustrate the existing information concerning the health effects of 2,4-, 2,6-, and Tg-DNT. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a “data need”. A data need, as defined in ATSDR’s *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments.

Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

3.12.2 Identification of Data Needs

With the exception of animal oral LD₅₀ values for 2,3- and 3,5-DNT, there are no data on the health effects of 2,3-, 2,5-, or 3,5-DNT in humans or animals by any route of exposure. As shown in [Figures 3-7](#) (2,4-DNT), [3-8](#) (2,6-DNT), and [3-9](#) (Tg-DNT), there are limited data on health effects in humans, primarily for Tg-DNT, following inhalation exposure.

The available reports generally lack quantitative information on exposure levels. Human data are particularly sparse. Most toxicity studies have focused on the main systemic effects of obvious clinical significance, as described in the previous sections.

The toxicity of these chemicals has been extensively investigated in animals after oral exposure, but not after inhalation exposure, and only in a very limited way after dermal exposure. The potential

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Figure 3-7. Existing Information on Health Effects of 2,4-DNT

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

Human

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

Animal

● Existing Studies

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Figure 3-8. Existing Information on Health Effects of 2,6-DNT

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

Human

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

Animal

● Existing Studies

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Figure 3-9. Existing Information on Health Effects of Technical-Grade DNT

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

Human

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

Animal

● Existing Studies

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carcinogenicity of these chemicals has been investigated following oral exposure in typical chronic bioassays as well as in less-than-life-time studies.

Acute-Duration Exposure. Although there are no human data available from acute-duration oral exposure to 2,3- 2,4-, 2,5-, 2,6-, or 3,5-DNT, data currently available from animal studies using single-dose exposure to 2,3- 2,4-, 2,5- and 2,6-DNT are appropriate for evaluation of oral toxicity (U.S. Army 1975, 1978a; Vernot et al. 1977). No data regarding the oral toxicity of 3,5-DNT were located. In gavage studies in rats and mice, LD₅₀ values of 1,120 and 1,070 mg/kg, respectively, were identified for 2,3-DNT (Vernot et al. 1977). The LD₅₀ values for 2,4-DNT determined after gavage dosing ranged from 270 to 650 mg/kg in rats and from 1,340 to 1,954 mg/kg in mice (U.S. Army 1975, 1978a; Vernot et al. 1977). Gavage dosing of 2,5-DNT identified LD₅₀ values of 710 mg/kg in rats and 1,230 mg/kg in mice (Vernot et al. 1977). After oral administration of 2,6-DNT, LD₅₀ values ranged from 180 to 795 mg/kg in rats and from 621 to 807 mg/kg in mice (U.S. Army 1975, 1978a; Vernot et al. 1977). Ataxia was observed in these animals before death. Decreased body weight gain was noted in rats administered 2,4-DNT via gavage at 398 mg/kg and observed for 48 hours and in rats administered 2,6-DNT at ≥ 50 mg/kg and observed for 48 hours; body weight loss occurred at 199 mg/kg 2,6-DNT (Deng et al. 2011). Slight cyanosis was observed in rats administered 60 mg/kg 2,4-DNT by gavage for 5 days (Lane et al. 1985), but no changes in hematological parameters were found in rats fed up to 273 mg/kg/day in the diet for 14 days (McGown et al. 1983). Evidence of erythrocytosis (increased red blood cells, hemoglobin, and hematocrit) was observed in rats administered 2,4-DNT at ≥ 99 mg/kg and in rats administered 2,6-DNT at 199 mg/kg at 24 and/or 48 hours after dosing (Deng et al. 2011). Hepatic effects, including increased blood cholesterol and alanine aminotransferase levels, and renal effects, such as hyaline droplet accumulation, were observed in rats fed 2,4-DNT in the diet for 14 days (McGown et al. 1983). Sinusoid congestion was noted in the livers of rats treated with 2,4-DNT at 398 mg/kg and in rats treated with 2,6-DNT at 199 mg/kg and evaluated at 24 or 48 hours; levels of albumin in the serum were also increased in rats treated with 2,4-DNT at 99 mg/kg (Deng et al. 2011). Data were insufficient to derive an acute-duration oral MRL for 2,6-DNT.

There were no acute-duration inhalation or dermal studies in humans available for evaluation. The LD₅₀ value determined after exposure to 2,6-DNT as an aerosol in rats (combined males and females) was 0.43 mg/L (0.24 mg/L for males and 0.66 mg/L for females) (CMA 1991). Treated rats showed evidence of respiratory distress (exaggerated breathing, ataxia, and lethargy); rats that died experienced lung congestion and increased relative lung weights. Both 2,4- and 2,6-DNT were shown to be mild primary

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dermal irritants in rabbits (U.S. Army 1975, 1978a). Additional acute inhalation studies and dermal studies would be useful for determining route-specific toxicity.

Intermediate-Duration Exposure. Currently available animal studies using repeated-dose exposure are appropriate for evaluation of oral toxicity for both 2,4- and 2,6-DNT (Ellis et al. 1985; Hazleton Laboratories 1977, 1982; Hong et al. 1985; Jones-Price et al. 1982; Lee et al. 1985; McGown et al. 1983; Smith et al. 1996; U.S. Army 1976, 1978b, 1979); no data were located regarding repeated-dose toxicity for 2,3-, 2,5-, or 3,5-DNT. Methemoglobinemia and its sequelae (Heinz bodies, anemia, reticulocytosis), hemosiderosis, extramedullary hematopoiesis, and cyanosis have been observed in animals after oral treatment with 2,4-, 2,6-, or Tg-DNT (Hazleton Laboratories 1977, 1982; U.S. Army 1976, 1978b). Mild hepatocellular dysplasia was observed in mice fed 2,4-DNT in the diet for 13 weeks (Hong et al. 1985; U.S. Army 1978b), but no hepatotoxicity was observed after 2,4-DNT administration to rats or dogs for the same duration (U.S. Army 1978a, 1978b). However, treatment with 2,6-DNT did cause bile duct hyperplasia in rats and mice (U.S. Army 1976). This lesion, as well as hepatic degeneration, was observed in dogs dosed with 2,6-DNT (U.S. Army 1976).

Oral administration of 2,4-DNT to rats, mice, or dogs for 13 weeks did not cause any significant adverse renal effects (Hong et al. 1985; U.S. Army 1978b). Administration of 2,6-DNT to dogs for 13 weeks caused renal inflammation and degeneration, which were not observed in rats or mice (U.S. Army 1976). Decreased body weight gain or weight loss was observed in rats and mice after administration of 2,4-DNT (Hong et al. 1985; Lee et al. 1985; Leonard et al. 1987; NCI 1978; U.S. Army 1978b, 1979) and in rats, mice, and dogs after administration of 2,6-DNT (U.S. Army 1976). Subchronic inhalation would be useful for determination of toxic effects in order to derive an MRL for 2,4-DNT and to determine a mechanism of action from routes of exposure that are more characteristic of occupational exposure.

Chronic-Duration Exposure and Cancer. There are no data available in humans regarding the carcinogenicity of 2,4- or 2,6-DNT. A retrospective cohort mortality study performed using data from workers at ammunition plants that used 2,4-DNT or Tg-DNT found no increases in mortality due to either malignant neoplasms as a whole or from particular cancers (Levine et al. 1986b). However, the small cohort examined in this study limited its statistical power. Both 2,4- and 2,6-DNT have been found to cause hepatocellular carcinoma in rats (Leonard et al. 1987; U.S. Army 1979). Renal cancer was observed in mice after administration of 2,4-DNT in the diet (U.S. Army 1979). EPA has derived a cancer slope factor for oral exposure to 2,4-DNT/2,6-DNT mixture (IRIS 2012).

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Excessive mortality rates from ischemic heart disease and residual diseases of the circulatory system were observed in ammunition plant workers (Levine et al. 1986a). Because it is expected that these workers would have a lower incidence of cardiovascular disease due to the healthy worker effect, this finding is unusual. Further epidemiological studies to verify these findings are needed; newer analyses should control for risk factors, particularly cigarette smoking, which was not done by the Levine et al. (1986a) study.

No data were located regarding the chronic toxicity of 2,3-, 2,5-, or 3,5-DNT. The currently available studies in laboratory animals on the effects of 2,4- and 2,6-DNT after chronic exposure are appropriate for evaluation of chronic oral toxicity (Ellis et al. 1985; Hazleton Laboratories 1982; Lee et al. 1985; Leonard et al. 1978; NCI 1978; U.S. Army 1978b, 1979). Hematological effects, including anemia, compensatory anemia, methemoglobinemia, and Heinz bodies, have been observed after chronic administration of 2,4-DNT to dogs, mice, and rats (Ellis et al. 1985; Hong et al. 1985; U.S. Army 1978b, 1979). Data were insufficient for the derivation of a chronic-duration oral MRL for 2,6-DNT. Severe hepatocellular changes, such as degeneration and vacuolation and dysplasia, were found in rats, mice, and dogs administered 2,4- or 2,6-DNT for chronic durations in oral exposure studies (Ellis et al. 1985; Hong et al. 1985; Leonard et al. 1987; U.S. Army 1979). Renal cystic dysplasia was observed in mice, but not rats or dogs, treated orally with 2,4-DNT for chronic-duration periods (Hong et al. 1985; U.S. Army 1979). Chronic-duration studies have not been performed in mice using 2,6-DNT to determine whether these findings would also result after administration of this isomer. Although no histopathological effects were found in adrenal, pituitary, or thyroid glands of rats after chronic oral administration of Tg-DNT, increases in parathyroid hyperplasia, fatty metamorphosis, and vascular ectasia were found (Hazleton Laboratories 1982). Further studies may be useful to verify these findings. Effects on body weight, including body weight loss, were reported in almost all chronic-duration oral studies (Ellis et al. 1985; Hazleton Laboratories 1982; Hong et al. 1985; Leonard et al. 1987; NCI 1978; U.S. Army 1979).

A well-controlled chronic inhalation study and dermal studies would be useful for determination of the potential for route-specific toxicity. In addition, for both 2,4- and 2,6-DNT, well-controlled epidemiological evaluations of larger occupationally exposed populations would contribute valuable insights regarding the human relevancy of chronic health effects observed in animal studies.

Genotoxicity. Both 2,4- and 2,6-DNT cause gene mutations in the reverse mutation assay using *S. typhimurium*. (Couch et al. 1981; Dellarco and Prival 1989; Spanggord et al. 1982b; Tokiwa et al. 1981; U.S. Army 1978a). However, the test system has given variable results because of the need for

3. HEALTH EFFECTS

metabolic activation and the sensitivity of the tester strains. *In vivo* assays using 2,4-DNT have shown UDS and S-phase synthesis using rat hepatocytes (Ashby et al. 1985; Mirsalis and Butterworth 1982; Mirsalis et al. 1989), chromosomal aberrations using human lymphocytes (Huang et al. 1995), and DNA binding in rat hepatocytes (Chadwick et al. 1993; La and Froines 1993). The genotoxicity of Tg-DNT is believed to be due to the potent genotoxicity of 2,6-DNT, as evidenced in an *in vivo-in vitro* hepatocyte UDS system (Mirsalis and Butterworth 1982). Both 2,4- and 2,6-DNT have induced DNA adducts in rat liver (La and Froines 1992, 1993). Studies currently available for 2,4- and 2,6-DNT are considered to be appropriate for evaluation of genotoxicity.

Reproductive Toxicity. The currently available laboratory data on reproductive toxicity are considered appropriate for evaluation of oral exposure of animals to both isomers. Several studies in rats, mice, and dogs with either isomer have shown impairment of the male reproductive system. The effects observed include testicular atrophy, degeneration of the seminal vesicles, and decreased sperm production (Bloch et al. 1988; U.S. Army 1976, 1978b, 1979). *In vitro* studies have shown that the testicular degeneration is due, at least in part, to structural changes in Sertoli cells (Reader and Foster 1990). Animal studies of reproductive toxicity using inhalation exposure would provide information relative to occupational exposure conditions.

Several assessments of reproductive function in exposed workers have been performed that did not detect differences in sperm production or fertility rates as a result of exposure (Hammill et al. 1982; Levine et al. 1985a; NIOSH 1982). However, an earlier study reported a significant reduction in the sperm counts of exposed workers, as well as an increase, of marginal statistical significance, in the number of spontaneous abortions in their wives (NIOSH 1980). These studies were all limited by the small exposure populations studied and the lack of historical individual exposure monitoring. Further epidemiological studies of larger exposed occupational populations with exposure data may be considered useful since questions of potential reproductive effects associated with these exposures have not yet been clearly resolved.

Developmental Toxicity. No data are available regarding developmental effects in humans after oral exposure to DNT, but animal studies that have been performed show possible developmental effects. The only developmental effect observed in a three-generation reproductive study in rats using 2,4-DNT was a decrease in pup viability. This decrease was attributed to maternal neglect and a high incidence of maternal death during parturition. Tg-DNT administered to pregnant dams caused a decrease in relative liver weight in postpartum pups and possible transient neurotoxicity (Jones-Price et al. 1982). Further studies may be useful to elucidate these effects. Additional animal studies using 2,4- and 2,6-DNT by

3. HEALTH EFFECTS

oral and inhalation routes should analyze fetal and maternal blood for hematological parameters. This is recommended because any factor that could reduce the amount of oxygen to developing tissue is expected to have adverse consequences in the offspring.

Immunotoxicity. Although no data are available regarding immunological or lymphoreticular effects in humans, some data on these end points are available in animals. The currently available information on the potential immunotoxic effects of 2,4- and 2,6-DNT is sufficient to describe the sensitizing potential of DNT. Mild sensitization has been reported in guinea pigs after dermal exposure to 2,6-DNT, but not 2,4-DNT (U.S. Army 1975, 1978a). No effects on IgE, the antibody associated with allergic or hypersensitive reactions, were reported in rats or dogs exposed to either the 2,4- or the 2,6-DNT isomer (Ellis et al. 1985; Lee et al. 1985; U.S. Army 1976, 1978b). Studies have not been performed that would describe effects on immunocompetence following exposure to DNT. A battery of immunotoxicity tests would provide a better assessment of possible effects in humans.

Neurotoxicity. The nervous system has been shown to be a major target of 2,4- and 2,6-DNT toxicity in animals (Ellis et al. 1985; Kozuka et al. 1979; Lee et al. 1979, 1985; U.S. Army 1979). Clinical signs in dogs have included incoordination and stiffness of the hind legs leading to complete paralysis; cerebellar vacuolation, hypertrophy, and focal gliosis; are cerebellar and brain stem hemorrhage. In mice, depression and hyperexcitability were observed, while some rats administered 2,6-DNT showed neuromuscular symptoms. More systematic examination of the neurological effects of these compounds in laboratory animals would be useful to assess fully behavioral abnormalities and morphological damage to the nervous system. Although results of an *in vitro* study in neuroblastoma cells show that 2,4-DNT is cytotoxic and produced cell death (Banerjee et al. 1999), the biochemical mechanisms of DNT neurotoxicity is not known.

Generalized symptoms of neurotoxicity, including headache, sleepiness, dizziness, and tingling pain in the extremities were reported in workers occupationally exposed to 2,4-DNT (McGee et al. 1947; Perkins 1919). However, the more recent occupational studies performed failed to examine workers for symptoms of neurotoxicity (Hammill et al. 1982; Levine et al. 1985a; NIOSH 1980, 1982). Because the early reports of potential neurotoxicity in exposed workers have not been followed-up in more recent studies, neurological examination of workers in occupational studies could provide additional information regarding the potential magnitude of neurotoxic effects.

3. HEALTH EFFECTS

Epidemiological and Human Dosimetry Studies. Epidemiology studies of workers exposed to DNT suggest a potential for heart disease in exposed populations (Levine et al. 1986a). Doses of DNT associated with heart disease in humans have not been determined. Further studies with historical cohort monitoring data and control for potentially confounding factors such as concomitant exposure to other chemicals and cigarette smoking would be useful to verify these findings.

Animal studies have indicated that the male reproductive system is a target of DNT toxicity. Epidemiological studies have provided only suggestive evidence of a reproductive effect in workers exposed to DNT. Studies of larger worker populations may help to determine more conclusively the magnitude of the potential for reproductive toxicity in exposed humans.

Other effects that were observed in animal studies but not confirmed in human populations include liver and kidney toxicity, neurotoxicity, and cancer. Well-controlled epidemiological studies examining these end points in humans would be useful.

Biomarkers of Exposure and Effect

Exposure. A rapid, accurate method for determining exposure to DNT has been developed using spectrophotometric analysis of complexes of primary arylamines, which result from the reduction of DNT and its metabolites (Smith et al. 1995).

Effect. Epidemiological studies that correlate quantitative estimates of exposure with disease outcomes would be useful. Studies that identify subtle physiological changes, such as altered blood chemistry indices, associated with a particular disease state are not available.

A disease registry is not currently available. The development of a registry of exposures and diseases would provide a useful reference tool for assessing the variations in exposure concentrations and health effects from, for example, geography, season, regulatory actions, presence of hazardous waste landfills, or manufacturing and use facilities. These assessments, in turn, would provide a better understanding of the needs for some types of research or data acquisition based on the current exposure concentrations.

Absorption, Distribution, Metabolism, and Excretion. The toxicokinetics of 2,4- and 2,6-DNT in rats by the oral route have been extensively studied. That Tg-DNT is absorbed and excreted in the urine by humans in an occupational setting, where the main routes of absorption are considered to be

3. HEALTH EFFECTS

inhalation and dermal, has also been documented. There are no data available in animals on the toxicokinetics of DNT by the dermal or inhalation routes. Toxicokinetics studies in rats administered the test materials by the inhalation and dermal routes would be critical in understanding possible differences in the toxicity of DNT by different routes of administration. The main routes of exposure of humans are dermal and inhalation. Understanding the possible differences in toxicity in animals by different routes would be valuable in determining the significance of findings to humans who may be exposed by inhalation or dermal routes.

Comparative Toxicokinetics. Absorption and excretion studies in several species indicate that there are considerable differences between mice and the other species evaluated. More detailed study of the metabolism of DNT by mice, including the role of biliary excretion and enterohepatic cycling, would assist in understanding why the metabolism in mice is different from other species and which species may be the most appropriate model for evaluating hazards and risks to humans.

Methods for Reducing Toxic Effects. The most important method for reducing the toxic effects of DNT is removal of the person from the area of exposure. Skin and eyes should be rinsed copiously (Bronstein and Currance 1994), although absorption through the skin has not been adequately examined. Gastric lavage, with subsequent administration of activated charcoal, and cathartics may be of some benefit in reducing peak absorption after oral exposure to DNT. Methylene blue treatment is used with patients presenting with serious methemoglobinemia (Ellenhorn 1997). No additional studies are considered necessary at this time to examine further methods for reducing body burden of DNT. Further studies on supportive therapy after DNT exposure, such as the use of hemodialysis, forced diuresis, hyperbaric oxygen, or hemoperfusion might be useful.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

There is inadequate experimental evidence to evaluate if the pharmacokinetics of DNT are different in children. There are no studies on whether DNT or its active metabolites can cross the placenta or be excreted in breast milk, so it cannot be determined if fetuses may be exposed *in utero* or if infants may be exposed via breast milk ingestion. There are also no data to show if DNT and its metabolites are stored in maternal tissues and thus might be later mobilized during gestation or lactation; however, DNT and its metabolites are not likely to be stored because of their low octanol-water partition coefficient.

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There is little experimental evidence to evaluate whether the metabolism of DNT or its mechanisms of action are different in children. As discussed in Section 3.7, newborns are highly sensitive to the methemoglobin-generating effect of DNT because of their deficiency in methemoglobin reductase (Gruener 1976), which reduces methemoglobin back to hemoglobin. In addition, newborns have a high concentration of fetal hemoglobin in their erythrocytes. It will be useful to determine if fetal hemoglobin is more sensitive to the methemoglobin-generating effect of DNT. It will also be helpful to have data on the metabolism and mechanism of action of DNT on children to determine if children are more vulnerable than adults to health effects from exposure to DNT, as some enzymes involved in DNT metabolism are known to have developmental regulation. There are no biomarkers of exposure or effect that have been validated in children or in adults exposed as children. There are no data to determine whether there are any interactions with other chemicals unique to children, or whether interactions observed in adults also occur in children. Although DNT is shown to be genotoxic, it is not known if parental exposure to DNT may affect children via parental germ cells, or if DNT may indirectly affect the fetus during maternal exposure.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

A follow-up to the Brüning et al. (1999, 2001) studies of copper miners exposed to DNT from explosives examining the possible association between Tg-DNT exposure and increased risk of urothelial cancer and kidney cancer is currently being conducted. No ongoing studies sponsored by NIH, NTP, or EPA were identified for DNTs.

4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of the individual isomers of DNT is located in [Table 4-1](#).

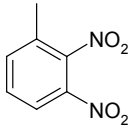
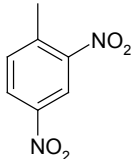
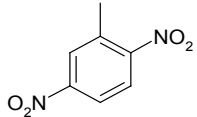
4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of the individual isomers of DNT is located in [Table 4-2](#). Data regarding specific isomers of DNT have been provided whenever possible. The isomers of DNT have many similar traits, including identical molecular weights, but also have distinguishable qualities. For instance, 2,4-DNT has higher melting and boiling points and a greater solubility in water than 2,6-DNT (HSDB 2012).

DNT is generally produced as a technical-grade mixture, which consists of approximately 76.5% 2,4-DNT and 18.8% 2,6-DNT. The remaining ~5% consists of other isomers of DNT and minor contaminants such as TNT and the mononitrotoluenes (HSDB 2012). Unspecified forms of DNT (DNT not otherwise specified or NOS) can be characterized under the CAS Registry Number of 25321-14-6. Where information pertains to Tg-DNT or DNT NOS, it has been so noted.

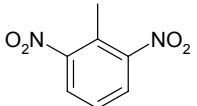
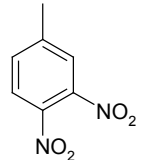
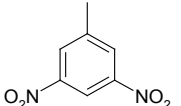
4. CHEMICAL AND PHYSICAL INFORMATION

Table 4-1. Chemical Identity of DNT

Characteristic	Information ^a		
Chemical name	2,3-Dinitrotoluene	2,4-Dinitrotoluene	2,5-Dinitrotoluene
Synonym(s)	1-Methyl- 2,3-dinitrobenzene; 2,3-DNT	1-Methyl- 2,4-dinitrobenzene; 2,4-dinitrotoluol; 2,4-DNT	2-Methyl- 1,4-dinitrobenzene; 2,5-DNT
Registered trade name(s)	No data	No data	No data
Chemical formula	C ₇ H ₆ N ₂ O ₄	C ₇ H ₆ N ₂ O ₄	C ₇ H ₆ N ₂ O ₄
Chemical structure			
Identification numbers:			
CAS registry	602-01-7	121-14-2	619-15-8
NIOSH RTECS	XT1400000	XT1575000	XT1750000
EPA hazardous waste	No data	U105	No data
OHM/TADS		7800118	
DOT/UN/NA/IMDG shipping	IMO 6.1 UN 1600 (molten) UN 2038 (solid or liquid)	IMO 6.1 UN 1600 (molten) UN 2038 (solid or liquid)	IMO 6.1 UN 1600 (molten) UN 2038 (solid or liquid)
HSDB	5499	1144	5504
NCI	No data	NCI-C01865	No data

4. CHEMICAL AND PHYSICAL INFORMATION

Table 4-1. Chemical Identity of Dinitrotoluenes

Characteristic	Information ^a		
Chemical name	2,6-Dinitrotoluene	3,4-Dinitrotoluene	3,5-Dinitrotoluene
Synonym(s)	1-Methyl- 2,6-dinitrotoluene; 2,6-DNT	4-Methyl- 1,2-Dinitrobenzene; 3,4-DNT	1-methyl- 3,5-dinitrobenzene; 3,5-DNT
Registered trade name(s)	No data	No data	No data
Chemical formula	C ₇ H ₆ N ₂ O ₄	C ₇ H ₆ N ₂ O ₄	C ₇ H ₆ N ₂ O ₄
Chemical structure			
Identification numbers:			
CAS registry	606-20-2	610-39-9	618-85-9
NIOSH RTECS	XT1925000	XT2100000	XT2150000
EPA hazardous waste	U106	No data	No data
OHM/TADS	8300219		
DOT/UN/NA/IMDG shipping	IMO 6.1 UN 1600 (molten) UN 2038 (solid or liquid)	IMO 6.1 UN 1600 (molten) UN 2038 (solid or liquid)	IMO 6.1 UN 1600 (molten) UN 2038 (solid or liquid)
HSDB	2931	5501	6463
NCI	No data	No data	No data

^aAll information obtained from HSDB 2012

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

4. CHEMICAL AND PHYSICAL INFORMATION

Table 4-2. Physical and Chemical Properties of DNT

Property	Information ^a		
Chemical name	2,3-Dinitrotoluene	2,4-Dinitrotoluene	2,5-Dinitrotoluene
Molecular weight	182.14	182.14	182.14
Color	Yellow	Yellow	No data
Physical state	Solid	Solid	Solid
Melting point	59–61 °C	71 °C	52.5 °C
Boiling point	284 °C ^b	300 °C (slight decomposition)	284 °C ^b
Density	No data	1.3208 (71 °C)	1.282 (111 °C)
Odor	No data	Slight	No data
Odor threshold:			
Water	No data	No data	No data
Air	No data	No data	No data
Solubility:			
Water	220 mg/L (25 °C, estimated) ^b	270 mg/L (22 °C)	220 mg/L (25 °C, estimated) ^b
Organic solvents	No data	Soluble in acetone, alcohol, benzene, ethanol, diethyl ether, pyridine, carbon disulfide	Soluble in ethanol; very soluble in carbon disulfide
Partition coefficients:			
Log K _{ow}	2.18 (estimated) ^b	1.98	2.18 (estimated) ^b
Log K _{oc}	No data	2.56 ^c	No data
Vapor pressure at 20 °C	3.97x10 ⁻⁴ torr (estimated) ^d	1.4x10 ⁻⁴ torr	3.97x10 ⁻⁴ torr (estimated) ^d
Henry's law constant at 25 °C			
Autoignition temperature	9.26x10 ⁻⁸ atm-m ³ /mol (estimated) ^b	5.4x10 ⁻⁸ atm-m ³ /mol ^e	9.26x10 ⁻⁸ atm-m ³ /mol (estimated) ^b
Flashpoint			
Flammability limits	No data	No data	No data
Conversion factors	No data	404 °F	404 °F
	No data	No data	No data
Explosive limits	1 ppm=7.40 mg/m ³ 1 mg/m ³ =0.13 ppm	1 ppm=7.40 mg/m ³ 1 mg/m ³ =0.13 ppm	1 ppm=7.40 mg/m ³ 1 mg/m ³ =0.13 ppm

4. CHEMICAL AND PHYSICAL INFORMATION

Table 4-2. Physical and Chemical Properties of Dinitrotoluenes

Property	Information ^a		
Chemical name	2,6-Dinitrotoluene	3,4-Dinitrotoluene	3,5-Dinitrotoluene
Molecular weight	182.14	182.14	182.14
Color	Yellow to red	Red	Yellow to red
Physical state	Solid	Solid	Solid
Melting point	66 °C	58.3 °C	93 °C
Boiling point	285 °C	284	315 °C ^b
Density	1.2833 (111 °C)	1.2594 (111 °C)	1.2772 (111 °C)
Odor	Slight	Slight	No data
Odor threshold:			
Water	No data	No data	No data
Air	No data	No data	No data
Solubility:			
Water	180 mg/L (20 °C)	100 mg/L (25 °C) ^f	145 mg/L (25 °C)
Organic solvents	Soluble in ethanol, chloroform	Soluble in ethanol and carbon disulfide; slightly soluble in chloroform	Soluble in benzene, ethyl ether, ethanol, chloroform, carbon disulfide
Partition coefficients:			
Log K _{ow}	2.10	2.08 ^g	2.18 (estimated) ^b
Log K _{oc}	No data	No data	No data
Vapor pressure at 20 °C	5.67x10 ⁻⁴ torr	3.97x10 ⁻⁴ torr (estimated) ^d	4.05x10 ⁻⁴ torr ^h
Henry's law constant at 25 °C			
Autoignition temperature	7.47x10 ⁻⁷ atm-m ³ /mol (estimated) ^b	9.26x10 ⁻⁸ atm-m ³ /mol (estimated) ^b	9.26x10 ⁻⁸ atm-m ³ /mol (estimated) ^b
Flashpoint			
Flammability limits	No data	No data	No data
Conversion factors	404 °F	404 °F	No data
	No data	No data	No data
Explosive limits	1 ppm=7.40 mg/m ³ 1 mg/m ³ =0.13 ppm	1 ppm=7.40 mg/m ³ 1 mg/m ³ =0.13 ppm	1 ppm=7.40 mg/m ³ 1 mg/m ³ =0.13 ppm

^aAll information obtained from HSDB 2012 unless otherwise stated.^bEPA 2011a^cU.S. Army 1980^dNeely and Blau 1985^eAltschuh et al. 1999^fChemicals Inspection Testing Institute 1992^gNakagawa et al. 1992^hAIChE 1989

4. CHEMICAL AND PHYSICAL INFORMATION

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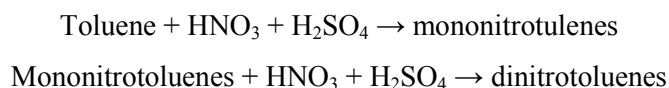
5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1 PRODUCTION

Tables 5-1 and 5-2 list the facilities in each state that manufacture or process 2,4- and 2,6-DNT, respectively; the intended use, and the range of maximum amounts of 2,4- and 2,6-DNT that are stored on site. The data listed in Tables 5-1 and 5-2 are derived from the Toxics Release Inventory (TRI10 2012). Only certain types of facilities were required to report; therefore, this is not an exhaustive list. In 2010, there were facilities located in 23 states that produced, processed or used 2,4-DNT and facilities located in 20 states that produced, processed, or used 2,6-DNT. The number of individual facilities and the amount produced on site varied in each state. The other isomers of DNT are not contained in the TRI database.

According to the 2011 Directory of Chemical Producers, 2,4- and 2,6-DNT were domestically manufactured by three corporations including Air Products and Chemicals, Inc. (production site: Pasadena, Texas), the BASF Corporation (production site: Geismar, Louisiana) and Bayer Material Science, LLC (production site: Baytown, Texas) (SRI 2011). No information regarding the other isomers of DNT was included in the directory. While no production volumes were reported, data collected by the EPA Inventory Update Reporting (IUR) system indicated that <500,000 pounds of 2,4-DNT were produced domestically in 2006 (EPA 2012a). The United Nations Screening Information Data Set (SIDS) Initial Assessment Report for Dinitrotoluene (mixed isomers) reported that the global production capacity of DNT is about 1.6 Mio t (1.6 million tons) annually (UNEP 2004).

2,4- and 2,6-DNT are generally produced as a mixture called Tg-DNT, which contains approximately 76.5% 2,4-DNT and 18.8% 2,6-DNT (with the remainder consisting of other isomers and minor contaminants such as TNT and mononitrotoluenes) (HSDB 2012). This mixture is commercially prepared in a two-step process. Toluene is nitrated with concentrated sulfuric and nitric acid resulting in the production of monoethanolamine (U.S. Army 1987). Monoethanolamine is then further nitrated to DNT in stirred tank reactors (EPA 1996b) as depicted below.



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

Table 5-1. Facilities that Produce, Process, or Use 2,4-DNT

State ^a	Number of facilities	Minimum amount on site in pounds ^b	Maximum amount on site in pounds ^b	Activities and uses ^c
AR	3	1,000	99,999	8, 12
CA	1	100	999	12
FL	3	1,000	999,999	5, 13
IA	3	1,000	99,999	1, 13, 14
IL	2	1,000	9,999	9, 12
IN	4	100	9,999,999	2, 3, 7, 9, 12
KY	4	1,000	999,999	12
LA	8	0	9,999,999	1, 3, 4, 6, 12, 14
MI	3	0	9,999	12
MO	1	10,000	99,999	8
MS	2	1,000	99,999	1, 5
NE	2	100,000	9,999,999	12
NJ	5	100	9,999,999	1, 3, 4, 6, 12
NV	2	0	99,999	6, 8, 12
NY	1	100	999	12
OH	2	1,000	9,999	12
OK	1	0	99	12
SC	1	10,000	99,999	12
TN	1	100,000	999,999	8
TX	9	1,000	9,999,999	1, 3, 4, 6, 9, 12
UT	1	1,000	9,999	12
VA	4	100,000	9,999,999	2, 3, 7
WV	1	100,000	999,999	1, 3, 6

^aPost office state abbreviations used.^bAmounts on site reported by facilities in each state.^cActivities/Uses:

- | | | |
|--------------------------|--------------------------|-----------------------------|
| 1. Produce | 6. Impurity | 11. Chemical Processing Aid |
| 2. Import | 7. Reactant | 12. Manufacturing Aid |
| 3. Onsite use/processing | 8. Formulation Component | 13. Ancillary/Other Uses |
| 4. Sale/Distribution | 9. Article Component | 14. Process Impurity |
| 5. Byproduct | 10. Repackaging | |

Source: TRI10 2012 (Data are from 2010)

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

Table 5-2. Facilities that Produce, Process, or Use 2,6-DNT

State ^a	Number of facilities	Minimum amount on site in pounds ^b	Maximum amount on site in pounds ^b	Activities and uses ^c
AR	1	1,000	9,999	12
CA	1	100	999	12
IN	2	100	999	2, 3, 12
KY	6	100	999,999	12
LA	4	100,000	999,999	1, 3, 4, 6
MI	3	0	9,999	12, 14
MS	1	1,000	9,999	1, 5
NV	2	10,000	99,999	6, 8, 12
OH	1	1,000	9,999	12
TN	2	100,000	999,999	8, 12
TX	5	1,000	999,999	1, 3, 4, 6, 12
VA	1	1,000	9,999	1, 5, 14
WV	3	10,000	999,999	1, 3, 6
AR	1	1,000	9,999	12
CA	1	100	999	12
IN	2	100	999	2, 3, 12
KY	6	100	999,999	12
LA	4	100,000	999,999	1, 3, 4, 6
MI	3	0	9,999	12, 14
MS	1	1,000	9,999	1, 5

^aPost office state abbreviations used.^bAmounts on site reported by facilities in each state.^cActivities/Uses:

- | | | |
|--------------------------|--------------------------|-----------------------------|
| 1. Produce | 6. Impurity | 11. Chemical Processing Aid |
| 2. Import | 7. Reactant | 12. Manufacturing Aid |
| 3. Onsite use/processing | 8. Formulation Component | 13. Ancillary/Other Uses |
| 4. Sale/Distribution | 9. Article Component | 14. Process Impurity |
| 5. Byproduct | 10. Repackaging | |

Source: TRI10 2012 (Data are from 2010)

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

5.2 IMPORT/EXPORT

In 1995, Rubicon, Inc. (Geismar, Louisiana), imported a total of 429,000 kg/year of Tg-DNT from an unknown location (EPA 1996).

5.3 USE

The most commercially important use of DNT is as a chemical intermediate in the production of toluene diisocyanate (TDI), a precursor to polyurethane polymers (HSDB 2012). DNT is hydrogenated to the toluenediamine that is then reacted with phosgene to yield TDI (EPA 1996b). It has been estimated that 99% of all DNT produced is used for this purpose (CMR 1983). Additionally, DNT is used in the production of TNT, as a plasticizer in propellants, and as a waterproofing, plasticizing, and gelatinizing agent in explosives (HSDB 2012). DNT is also used as an intermediate in the production of dyes and as a modifier for smokeless powders in the munitions industry (HSDB 2012). 2,4-DNT itself is used in the air bags of automobiles (Ellenhorn 1997).

5.4 DISPOSAL

2,4-DNT and 2,6-DNT are listed as toxic substances under Section 313 of the Emergency Planning and Community Right to Know Act (EPCRA) under Title III of the Superfund Amendments and Reauthorization Act (SARA) (EPA 1995). Disposal of wastes containing DNT is controlled by a number of federal regulations (see Chapter 8).

Limited information is available regarding the appropriate disposal of DNT. NIOSH recommends that small quantities be swept onto paper or other flammable material and incinerated in a suitable combustion chamber. Larger quantities should be reclaimed; if this is not practical, then they should be dissolved in fuel oil and atomized in a suitable combustion chamber (HSDB 2012). The ultimate disposal of DNT can be achieved by controlled incineration in an incinerator unit equipped with an alkaline scrubber (HSDB 2012). DNT has also been proposed as a potential candidate for rotary kiln incineration at 820–1,600 °C or fluidized bed incineration at 450–980 °C, with residence times of seconds for gases and liquids and longer for solids (HSDB 2012).

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

DNT (2,4- and 2,6-DNT) has been identified in at least 98 of the 1,699 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2007). However, the number of sites evaluated for DNT is not known. The frequency of these sites can be seen in [Figure 6-1](#).

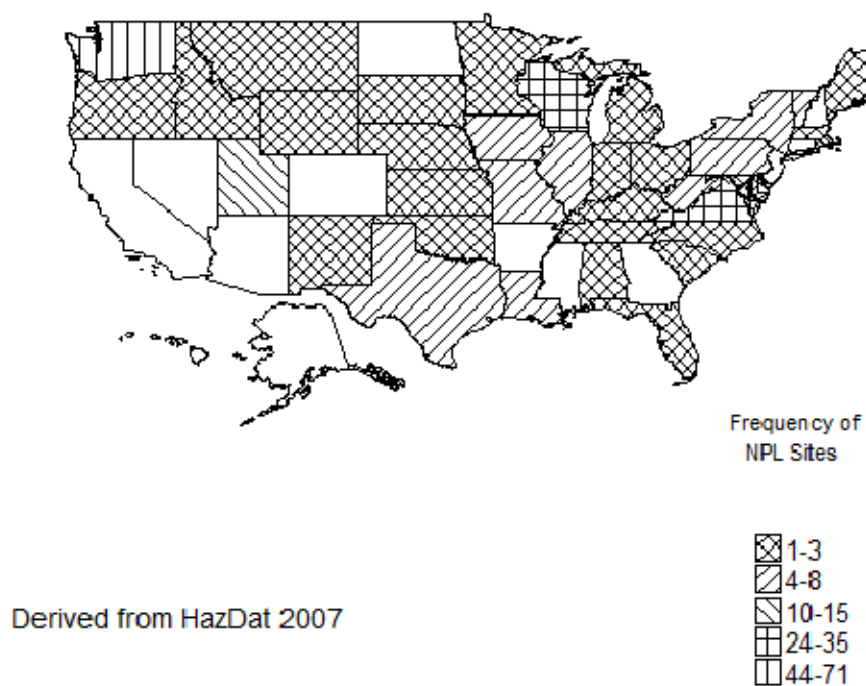
The available data provide a complex and incomplete view of the overall potential for human exposure to isomers of DNT. Little direct knowledge of the magnitude of environmental exposure pathways exists. Data regarding exposure of humans DNT have been obtained primarily from the workplace.

DNT has been found in waste water and groundwater in and around munitions sites (Jenkins et al. 1986), and 4-nitrotoluene and dinitrobenzene, structural analogues of DNT, are taken up by plants (McFarlane et al. 1987). However, predictions of environmental exposure pathways based on measurements of structural analogues of DNT are severely limited by the complex abiotic reactions of DNT in the environment and by the different pathways, rates, and products of biological reduction and/or oxidation of 2,4- and 2,6-DNT.

The relatively low log octanol-water partition coefficients ($\log K_{ow}$) of the DNT isomers (1.98–2.18) suggest that DNT released to the environment would not bioaccumulate. Measured bioconcentration factors also indicate that DNT is not expected to bioaccumulate in fish and other aquatic species (Lang 1997; NITE 2002). DNT is not highly adsorbed to soil or sediment and may leach from the soil surface to groundwater. DNT is degraded by oxidation, photolysis, and biotransformation in water or soil, but a variety of degradation products, about which very little is known, are formed. Volatilization is expected to occur slowly from water and soil surfaces and the rate of hydrolysis is negligible.

Studies of occupational exposures to DNT indicate that inhalation and dermal contact can result in absorption of DNT into the body. The general population is not expected to be exposed to DNT unless they reside near a source area, in which case, dermal contact and incidental ingestion pathways are the likely routes of exposure.

6. POTENTIAL FOR HUMAN EXPOSURE

Figure 6-1. Frequency of NPL Sites with 2,4 and 2,6 DNT Contamination

6. POTENTIAL FOR HUMAN EXPOSURE

6.2 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ 10 or more full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes $\geq 25,000$ pounds of any TRI chemical or otherwise uses $>10,000$ pounds of a TRI chemical in a calendar year (EPA 2005).

Section 112 of the Clean Air Act (CAA) lists 2,4-DNT as one of the original hazardous air pollutants (HAPs) known to cause, or suspected of causing, cancer or other serious human health effects or ecosystem damage (EPA 2000b). EPA's National Emission Inventory (NEI) database collects information about sources that emit criteria air pollutants and their precursors, and HAPs for the 50 United States, Washington DC, Puerto Rico, and the U.S. Virgin Islands (prior to 1999, criteria pollutant emission estimates were maintained in the National Emission Trends [NET] database and HAP emission estimates were maintained in the National Toxics Inventory [NTI] database). The NEI database derives emission data from multiple sources including state and local environmental agencies; the TRI database; computer models for on-road and off-road emissions; and databases related to EPA's Maximum Achievable Control Technology (MACT) programs to reduce emissions of hazardous air pollutants. Using composite data from the NTI database from 1990 to 1993, it was estimated that the annual emissions of 2,4-DNT in the United States was approximately 3.5 tons per year during that time frame (EPA 2000b). Data downloaded from the 2005 NEI indicated that the total emission of 2,4-DNT was approximately 4 tons, with the biggest source arising from nonpoint source waste disposal.

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6.2.1 Air

Estimated releases of 13,039 pounds (~5.9 metric tons) of 2,4- and 2,6-DNT to the atmosphere from 6 domestic manufacturing and processing facilities in 2010, accounted for about 98% of the estimated total environmental releases from facilities required to report to the TRI (TRI10 2012). These releases are summarized in [Tables 6-1](#) and [6-2](#). The TRI database does not contain information regarding the other isomers of DNT.

The Great Lakes Regional Air Toxic Emissions Inventory Project estimated that 1,894 pounds of 2,4-DNT were emitted to the air of the Great Lakes watershed in 2002 (Great Lakes Commission 2006). The overwhelming majority of these emissions arose from local point and area sources such as production or use facilities. No data were available for the other isomers of DNT.

Minute amounts of nitrotoluene are formed by the photochemical reaction of toluene, nitrogen oxides, and sunlight (Atkinson et al. 1980). Although DNT could be formed subsequently, it would be subject to photolysis and would not be likely to accumulate enough to contribute significantly to human exposure.

DNT was identified in air samples at one current or former NPL site where it was detected in some environmental media (HazDat 2007).

6.2.2 Water

According to the Toxics Release Inventory, in 2010, there were no reported releases of DNT to water (TRI10 2012). This is not an exhaustive list, however, since only certain types of facilities are expected to report. The detection of DNT in water has been reported (Feltz et al. 1990; Shackelford and Keith 1976; Staples et al. 1985). Both 2,4- and 2,6-DNT are recognized as major components in waste waters from TNT manufacturing facilities (Spanggord and Suta 1982; Spanggord et al. 1982a). DNT occurs in samples of TNT waste waters at concentrations of 0.04–48.6 mg/L (2,4-DNT) and 0.06–14.9 mg/L (2,6-DNT). The occurrence of DNT in waste waters from other manufacturing uses (e.g., polyurethane forms) has not been reported. The frequency of detection of DNT in surface waters, as indicated in the STORET database (Staples et al. 1985), is low. Slightly over 1% of the stations reported detectable quantities of DNT, and the median of positive samples was <10 µg/L. The presence of DNT was not detected in samples of sediment or biota.

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Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use 2,4-DNT^a

Reported amounts released in pounds per year ^b									
State ^c	RF ^d	Air ^e	Water ^f	UI ^g	Land ^h	Other ⁱ	Total release		
							On-site ^j	Off-site ^k	On- and off-site
IA	1	1,438	0	0	0	0	1,438	0	1,438
IN	1	0	0	0	0	0	0	0	0
KY	1	9,000	0	0	0	0	9,000	0	9,000
LA	1	0	0	0	0	0	0	0	0
OH	1	0	0	0	46	0	0	46	46
VA	1	0	0	0	0	130	0	131	131
Total	6	10,438	0	0	46	130	10,438	177	10,615

^aThe TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.

^bData in TRI are maximum amounts released by each facility.

^cPost office state abbreviations are used.

^dNumber of reporting facilities.

^eThe sum of fugitive and point source releases are included in releases to air by a given facility.

^fSurface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).

^gClass I wells, Class II-V wells, and underground injection.

^hResource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.

ⁱStorage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown

^jThe sum of all releases of the chemical to air, land, water, and underground injection wells.

^kTotal amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI10 2012 (Data are from 2010)

6. POTENTIAL FOR HUMAN EXPOSURE

Table 6-2. Releases to the Environment from Facilities that Produce, Process, or Use 2,6-DNT^a

State ^c	RF ^d	Reported amounts released in pounds per year ^b						Total release	
		Air ^e	Water ^f	UI ^g	Land ^h	Other ⁱ	On-site ^j	Off-site ^k	On- and off-site
IN	1	1	0	0	0	0	1	0	1
KY	1	2,600	0	0	0	0	2,600	0	2,600
OH	1	0	0	0	25	21	0	46	46
Total	3	2,601	0	0	25	21	2,601	46	2,647

^aThe TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.

^bData in TRI are maximum amounts released by each facility.

^cPost office state abbreviations are used.

^dNumber of reporting facilities.

^eThe sum of fugitive and point source releases are included in releases to air by a given facility.

^fSurface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).

^gClass I wells, Class II-V wells, and underground injection.

^hResource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.

ⁱStorage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown

^jThe sum of all releases of the chemical to air, land, water, and underground injection wells.

^kTotal amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI10 2012 (Data are from 2010)

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Hashimoto et al. (1982) reported the release of as much as 150 kg/day of DNT isomers (2,4-, 2,6-, 2,5-, 2,3-, and 3,4-DNT) in the effluent from a single coastal industrial drain in Dokai Bay, Japan, with a daily average release of 76 kg/day over 7 months. However, DNT concentrations in the bay decreased with distance from the site more rapidly than predicted by simple dilution and tidal action. The salting-out effect of sea water on nitroaromatic compounds was probably responsible for this effect (Hashimoto et al. 1984).

DNT was identified in groundwater samples at 99 current or former NPL sites where it was detected in some environmental media (HazDat 2007). DNT was identified in surface water samples at 40 current or former NPL sites where it was detected in some environmental media (HazDat 2007).

6.2.3 Soil

Estimated releases of 71 pounds (~0.0265 metric tons) of 2,4- and 2,6-DNT to soils from 6 domestic manufacturing and processing facilities in 2010, accounted for about 0.5% of the estimated total environmental releases from facilities required to report to the TRI (TRI10 2012). The TRI database does not contain information regarding the other isomers of DNT. There were no reported releases to underground injection wells. These releases are summarized in [Tables 6-1](#) and [6-2](#).

The extensive use of DNT as an intermediate in the synthesis of toluenediisocyanate and polyurethane foam is not a reported source of releases to soil. However, DNT can be a contaminant in the soil from explosives, propellants, etc. Residues of DNT were observed to be deposited onto the surface of military live-fire training soil (Jenkins 2006). As a result, mean surface soil concentrations ranged from <0.001 to 84 mg/kg for 2,4-DNT and from <0.001 to 4.6 mg/kg for 2,6-DNT (Jenkins 2006).

DNT was identified in soil samples at 87 current or former NPL sites where it was detected in some environmental media (HazDat 2007). DNT was identified in sediment samples at 25 current or former NPL sites where it was detected in some environmental media (HazDat 2007).

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6.3 ENVIRONMENTAL FATE**6.3.1 Transport and Partitioning**

The water solubilities of DNT are moderate (Callahan et al. 1979), and the octanol-water partition coefficients are low (EPA 1982c). As a result, there is a potential for DNT to leach from soil into groundwater. U.S. Army (1980) determined partitioning of 2,4-DNT onto environmental media and found that the soil organic carbon partition coefficient (K_{oc}), the octanol-water partition coefficient (K_{ow}), and the partition bioconcentration factor (K_b) were 364, 2, and 64, respectively. EPA (1982c) calculated sediment-water partitioning coefficients of 45 and 92 for 2,4- and 2,6-DNT, respectively. Depending on the nature of the sediment load, the total concentration of DNT carried in the soil and water column could be high. DNT in buried munitions wastes could potentially be released to groundwater or transported as contaminated soil and sediment. The low Henry's Law constants for the isomers of DNT (see Table 4-2) suggest that volatilization from water and moist soil surfaces will occur slowly. U.S. Army (1980) used experimentally determined volatilization rate constants of 2,4-DNT obtained under laboratory conditions, to extrapolate a volatilization rate constant for 2,4-DNT in an environmental aquatic water body with a depth of approximately 6.5 feet and ambient temperature of about 20 °C. Using this extrapolated rate constant, a volatilization half-life of >400 days is estimated for 2,4-DNT.

Steady-state whole-fish bioconcentration factors (BCFs) of 2,4-DNT measured in carp during two different equilibrium periods were 9.15 and 4.15 (Lang et al. 1997). BCF values of DNT (mixed isomers CAS Registry Number 25321-14-6) measured in carp over a 6-week incubation period were 0.6–2.9 at a nominal concentration of 0.25 ppm and 3.2–21.2 at a nominal concentration of 0.025 ppm (NITE 2002). BCF values of <2.7 were also measured for 3,4-DNT in carp over a 6-week incubation period (NITE 2002). These data suggest that bioaccumulation in fish and other aquatic organisms will be low.

Direct measurement of plant uptake of DNT has not been made, but plant uptake is predicted to occur based on its low octanol-water partition coefficient. Structural analogy with 1,3-dinitrobenzene and 4-nitrotoluene (McFarlane et al. 1987) suggests that 2,4- and 2,6-DNT would be readily taken up by plants. Plant uptake of related nitroaromatic compounds such as 2,4,6-TNT and its byproduct, 4-amino-2,6-DNT, has also been observed and is inversely proportional to soil organic carbon content (Pennington 1988). The relative concentrations in the plants was root > stem > leaves > seed and food (U.S. Army 1990). Root uptake by plants can be inhibited by the increase of sorption to the soil. Sorption can reduce the bioavailability of organic compounds to target organisms. In a bioavailability and phytotoxicity study, the toxicity of 2,4-DNT to the aquatic duckweed plant was significantly reduced by sorption of

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DNT to potassium smectite clay (Roberts 2007). In general, 2,4-DNT is expected to adsorb to suspended soil and sediment in water based on its K_{oc} . Nitroaromatic compounds adsorb to the soil mainly by forming electron-donor acceptor complexes with clays (Haderlein 1996). In anoxic conditions, sorption of the tested aromatic compounds followed the order of DNT>ANT>DATs, where monoamines (ANT) and diaminotoluenes (DAT) were the derivatives of DNT (Yang 2008).

The toxicity of the transformation products of 2,6-DNT was studied by Nipper et al. (2005) in spiked sandy, fine-grained marine sediments and in seawater. In environments with the spiked sediments, toxicity to the micro-algae, *Ulva fasciata*, decreased as 2,6-DNT was biotransformed to 2-amino-6-nitrotoluene. Subsequent studies indicated that toxicity to the copepod *Schizopera knabeni* increased with biotransformation. Therefore, 2-amino-6-nitrotoluene was more toxic than 2,6-DNT to the copepod, and less toxic to the micro-algae. Likewise, in environments with the spiked seawater tests, 2-amino-6-nitrotoluene was also more toxic than its parent compound to the copepod, but not to the micro-algae. These studies indicate that toxicity to the 2,6-DNT degradation products vary with the environment and species.

The Pre-Biologic Screen (PBS) model for ecotoxicologic effects (Gillett 1983) estimates a score (heavy concern, concern, or no concern) for a compound determined by the octanol-water partition coefficient, the Henry's Law constant, and the half-life in the medium of interest. The score indicates the compound's potential for (a) bioaccumulation and multi-media/multispecies effects, (b) bioaccumulation and long-term effects, (c) persistence and interactions in the water column, including plant uptake and leaching, and (d) direct and indirect effects in the atmosphere (e.g., smog formation, plant fumigation, stratospheric modification). Both 2,4- and 2,6-DNT are of concern or heavy concern only for (c), persistence and interactions in the water column, depending on the value used for half-life. Since the degradation of DNT is so dependent on environmental conditions and the presence of effective microorganisms, the protective view that DNT is of heavy concern for persistence in water, plant uptake, and leaching to groundwater may be warranted. The lack of concern for bioaccumulation, multimedia/multispecies action, and atmospheric action also appears to be justified.

6.3.2 Transformation and Degradation

6.3.2.1 Air

Based on its rapid photolysis in water, DNT is presumed to be subject to oxidation of its methyl group, decarboxylation, ring oxidation, and/or nitroreduction in air and sunlight. DNT is slowly degraded in the

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atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life in air is estimated to be approximately 50 days for the DNT isomers (EPA 2011a).

6.3.2.2 Water

DNT may be degraded in water through several mechanisms, including photolysis, microbial biodegradation, ozonation and chlorination, and oxidation by strong oxidants such as hydrogen peroxide, ozone, or oxone (Bausum et al. 1992; Bradley et al. 1995; EPA 1979; Freedman et al. 1996; Ho 1986; Noguera and Freedman 1996; U.S. Navy 1977). Ho (1986) studied photooxidation of 2,4-DNT in aqueous solution in the presence of hydrogen peroxide and suggested that the following degradation pathway of 2,4-DNT: 2,4-DNT \rightarrow 1,3-dinitrobenzene \rightarrow hydroxynitrobenzene derivatives \rightarrow carboxylic acids \rightarrow CO₂, H₂O, and HNO₃. Oxidation of aqueous DNT with hydrogen peroxide or ultraviolet (UV) irradiation alone was very slow, and elimination was not complete. Dillert et al. (1995) reported that degradations of DNT and several other nitroaromatics were accelerated in irradiated TiO₂ suspensions and that the degradation rates were dependent solution pH and light intensity. At given temperature, pH, and photo intensity, degradation rates were shown to decrease in order of 2-nitrotoluene > nitrobenzene > DNT > 1,3-dinitrobenzene > TNT > trinitrobenzene, and the degradations followed first-order kinetics. The photocatalytic oxidation of 2,6-DNT in aqueous suspension of TiO₂ produces ammonium and nitrate ions as the predominant species (Kumar and Davis 1997).

The presence and potential toxicity of DNT in waste water have spurred considerable study of the abiotic and biotic fate of 2,4- and 2,6-DNT. U.S. Army (1980) reported that the half-lives of DNT in three sunlit natural waters were 3–10 hours, whereas the photolysis half-life in distilled water was 43 hours. At different latitudes and sunlight conditions, they estimated that the photolysis half-life in waters would range from approximately 1.8 days (summer sunlight conditions) to 11.5 days (winter conditions). Simmons and Zepp (1986) found that dissolved or suspended humic substances greatly enhance (10–17 times) indirect photolysis of nitroaromatic compounds with a nitro group ortho to a methyl group.

DNT may be also degraded by ozonation and chlorination. Lee and Hunter (1985) reported that both ozone and chlorine produced <17% reduction of 2,6-DNT, whereas 2,4-DNT was more vulnerable, yielding about 35% reduction by chlorine and 60% reduction by ozone. Contact time did not appear to have any impact on the reduction rates.

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Studies have shown that DNT can be used as a sole carbon source for microorganisms commonly detected in natural waters. Using bay water obtained near an Army munitions facility as inoculum, >90% degradation of 2,4-DNT was observed after 6 days following a 2–3-day lag period (U.S. Army 1980). Under anaerobic conditions, the half-life of 2,6-DNT in non-acclimated sewage was found to be 28 days, with no loss of the compound under aerobic conditions during the same period (Hallas and Alexander 1983). Liu et al. (1984) reported complete biotransformation of 2,4-DNT within 14 days under anaerobic conditions using a fresh sample of activated sludge diluted with distilled water as inoculum. The intermediates of biotransformation were identified as 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2-nitroso-4-nitrotoluene, and 4-nitroso-2-nitrotoluene. Parrish (1977) investigated 190 fungal species from 98 genera, but found only 5 capable of 2,4-DNT biotransformation. Valli et al. (1992) reported degradation of 2,4-DNT as the sole source of carbon and energy by the lignin-degrading fungus *Phanerochaete chrysosporium* under aerobic conditions, resulting in stoichiometric release of nitrate.

Several additional studies have shown biodegradation of DNT from microorganisms isolated from areas that are frequently exposed to DNT and other structurally similar compounds such as TNT (Bausum et al. 1992; Bradley et al. 1995; Freedman et al. 1996). Bausum et al. (1992) found complete degradation of 20 ppm 2,4-DNT and 20 ppm 2,6-DNT in water samples taken downstream a short distance from the Radford Army Ammunition Plant in Radford, Virginia. A lag time was noted prior to the breakdown for both of the two compounds with 2,4-DNT exhibiting the shorter lag time. Microbial enrichment cultures were developed from the collected water samples by exposing the cultures to increasing concentrations of 2,4- and 2,6-DNT. Degradation and visible turbidity in the suspension medium were noted up to a level of 130 ppm. In a separate but related study, degraded DNT was shown to be converted to CO₂ with 2,4-DNT conversion occurring at a greater rate than that of 2,6-DNT; concentrations ranged from 0.004 to 10.0 ppm (Bausum et al. 1992). The rate of mineralization to CO₂ was concentration dependent and increased with increasing concentration.

In the Bradley et al. (1995) study, a culture of microorganisms taken from aquifer sediments at an explosives-contaminated site was observed to be capable of removing added 2,4- and 2,6-DNT. The removal rate of 2,4-DNT after 70 days was >99% after application of 100 µM of 2,4-DNT. Removal of 2,6-DNT at the same concentration was less efficient, with 60% of the compound being removed after 70 days. Breakdown products from 2,4-DNT degradation included 4-amino-2-nitrotoluene and 2-amino-4-nitrotoluene. Carbon dioxide was released during the degradation process. Aminonitrotoluene isomers were also detected as breakdown products of a solution of 2,4-DNT and ethanol (Freedman et al. 1996). The Freedman et al. (1996) study exposed an inoculum from a wastewater treatment plant at an

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ammunition plant to a solution of 2,4-DNT and ethanol and a solution of 2,4-DNT and ether. The concentration of 2,4-DNT at each application was 0.55 mM and the concentrations of ethanol and ether were 600 and 142 mg/L, respectively. Ethanol and ether were chosen because they are often found in munitions manufacturing waste-water streams along with 2,4-DNT. As stated above, aminonitrotoluene isomers were detected as products of the solution containing 2,4-DNT and ethanol. The degradation of ethanol was believed to be driving a partial reduction of 2,4-DNT before the oxidation of 2,4-DNT took place. In contrast, ether at the applied concentration slowed the rate of 2,4-DNT degradation. Low chemical oxygen demand during the studies suggests that DNT was mineralized to a significant degree.

Spanggord et al. (1991) studied the degradation pattern of 2,4-DNT using, water samples obtained from the Waconda Bay located near a TNT manufacturing facility (Volunteer Army Ammunition Plant) in Chattanooga, Tennessee. Samples were enriched with DNT and incubated for 5 days. The *Pseudomonas* sp. strain aerobically degraded DNT as a sole source of carbon, and the nitro groups were oxidatively removed without prior reduction to the amines. Since this *Pseudomonas* sp. strain used DNT as a carbon source, it may be useful for the removal of other nitrotoluenes from contaminated soils or from industrial waste streams. Likewise, it may also be useful since the oxidative removal of the nitro groups bypasses the accumulation of toxic amino derivatives.

The co-metabolism of DNT with ethanol, methanol, and acetic acid has been studied under anaerobic conditions (Cheng et al. 1995). High concentrations of ethanol accelerated the reductive transformation of 2,4-DNT by supporting the growth of the anaerobic bacteria. The rate of 2,4-DNT biotransformation was much higher with the addition of ethanol than with the addition of methanol or acetic acid in anaerobic conditions (Cheng et al. 1995). In a culture using a continuous flow laboratory fermentor under anaerobic conditions with both 2,4-DNT and ethanol as substrates, 2,4-DNT was completely transformed to 2,4-diaminotoluene (Cheng et al. 1996). During the biotransformation, two intermediates were formed: 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene.

The products formed from anoxic biotransformation of 2,4-DNT by two denitrifying enrichment cultures with ethanol provided as a primary substrate were characterized in one study (Noguera and Freedman 1997). One culture was developed with inoculum acclimated to DNT, the other with activated sludge that was not routinely exposed to nitroaromatic compounds. The acclimated culture consumed DNT twice as fast as the unacclimated culture, with reduction of DNT to aminonitrile toluenes as the initial pathway. The principal metabolites identified in the acclimated culture were 6-nitroindazole, 2-nitrotoluene, and 4-nitrotoluene, as well as products from acetylation at the *para* position (4-acetamide-2-nitrotoluene and

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4-acetamidetoluene). Reduction of aminonitrotoluenes to 2,4-diaminotoluene also occurred, and its subsequent disappearance results in accumulation of significant amount of nonfilterable material in both cultures. The soluble metabolites formed from the unacclimated culture were more hydrophilic. Initial characterization of the highly hydrophilic metabolites indicated approximately equal amounts of negatively-charged and neutral compounds.

Biotransformation of DNT by a *Pseudomonas aeruginosa* strain, which was isolated from the waste water of the Radford Army Ammunition Plant (RAAP) located in Radford, Virginia was observed under both aerobic and anoxic conditions (Noguera and Freedman 1996). The biotransformation was mainly reductive under both of these conditions and was reflective of the cometabolic transformations that can occur in the presence of easily degradable organic matter such as ethanol. *P. aeruginosa* reduced both nitro groups of DNT resulting in the primary breakdown products of 4-amino-2-nitrotoluene and 2-amino-4-nitrotoluene, with small amounts of 2,4-diaminotoluene also formed. Several DNT metabolites from acetylation of the arylaminos were also identified, including 4-acetamide-2-nitrotoluene, 2-acetamide-4-nitrotoluene, 4-acetamide-2-aminotoluene, and 2,4-diacetamidetoluene.

Clostridium acetobutylicum has received interest in anaerobic TNT bioremediation systems due to their ability to rapidly reduce aryl nitro groups. Results indicated that dihydroxylaminotoluenes are the predominant primary intermediates in the transformation of the DNT isomers by *C. acetobutylicum* and that further metabolism of the dihydroxylaminotoluenes in cell cultures and extracts resulted in the formation of arylamines through hydroxylamine reduction (Hughes 1999).

6.3.2.3 Sediment and Soil

Microorganisms indigenous to surface soils collected at a munitions-contaminated site were reported to transform 2,4- and 2,6-DNT to amino-nitro intermediates within 70 days (Bradley et al. 1994). Another study showed that composting can decrease the concentrations of explosives, such as TNT, in contaminated soil, but neither 2,4- nor 2,6-DNT was detected in the compost (Griest et al. 1993). A study of soil sample handling times indicated that lower temperatures retard the breakdown of 2,4-DNT (Grant et al. 1995). 2,4-DNT was observed to be more stable than TNT in contaminated soils (Grant et al. 1995).

DNT was not readily biodegradable using a standardized Organisation for Economic Co-operation and Development (OECD) test. 3,4-DNT and mixed isomers of DNT (CAS Registry Number 25321-14-6) achieved 0% of their theoretical biochemical oxygen demand (BOD) over a 14-day incubation period

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using an activated sludge inoculum and the modified MITI (OECD 301 C) test (NITE 2002). The DT_{50} of 2,4-DNT in an organic soil (82% sand, 13% silt, 5.1% clay, 2.3% organic carbon) was reported as 7 days and the DT_{90} was 191 days (UNEP 2004); however, other studies cited in this report indicated limited degradation of DNT under environmental conditions. In a test system maintained under anaerobic conditions, performed according to EPA-Guideline No. 796.3140, 0% biodegradation was observed within 56 days (UNEP 2004).

In a study conducted by Nishino (1999), the mineralization of 2,4- and 2,6-DNT was examined in soil slurries. DNT-degrading bacteria cultures commonly used in contaminated water systems were examined to see if they were able to degrade DNT in contaminated soils. It was found that microorganisms indigenous to the soils did not convert DNT to aminonitrotoluenes during the short incubation times required to mineralize DNT. DNT-degrading bacteria, however, removed over 99% of the initial DNT in the soil after 2–3 days of incubation. Disappearance of DNT was accompanied by $^{14}CO_2$ release and stoichiometric appearance of nitrite.

In another study, soil from a former ammunition plant was anaerobically treated in a laboratory slurry reactor. 2,4-DNT was completely reduced to undetectable levels in the reactor. The contaminated soil was also tested in a larger technical scale where the soil slurry was treated anaerobically and subsequently aerobically to complete the bioremediation process. An overall reduction of > 99% of the contaminants was observed, and ecotoxicological tests showed that the toxicity of the soil could not be detected after the anaerobic/aerobic process (Lenke et al. 1998).

The biotransformation of 2,4- and 2,6-DNT by the indigenous microflora contained in marine sediment from a shipwreck site near Halifax Harbour was studied by Yang et al. (2008). Incubation of 2,4- and 2,6-DNT in anaerobic sediment-water slurries at 10 °C led to the disappearance of both 2,4- and 2,6-DNT and their reduction to their monoamine derivatives in 10 days. The derivatives were progressively reduced even further to 2,4- and 2,6-diaminotoluenes in a series of 50 days for 2,4-DNT and 35 days for 2,6-DNT.

The biodegradation of sorbed 2,4-DNT by the *Burkholderia sp.* strain was examined in a clay-rich aggregated porous medium by Ortega-Calvo et al. (1999). *Burkholderia sp.* exhibited sensitivity to the cold and was unable to complete the degradation process at low temperatures (Monti 2005). Therefore, *P. fluorescens* was genetically modified (by intergeneric transformation of the DNT genes from the *Burkholderia sp.* strain to the *P. fluorescens* strain) to use 2,4-DNT as the sole nitrogen source to

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completely degrade the compound (Monti 2005). *P. fluorescens* was shown to be capable of degrading 2,4-DNT in temperatures as low as 10 °C and significantly decreased the toxic effects of 2,4-DNT on specific plants.

The correlation between the mutagenicity and biodegradability of TNT and its analogs, including 2,4- and 2,6-DNT, was reported in Maeda et al. (2007). The umu test using luminescent bacteria was employed to assess the mutagenicity and biodegradability of the nitroaromatic compounds. The *Pseudomonas sp.* strain TM15 bacteria isolated from TNT contaminated soils in Japan was found to biotransform the aromatic compounds harboring three nitro groups efficiently, although 2,4- and 2,6-DNT had low degradation rates. It was demonstrated that the mutagenicity, induced by the nitroaromatic compounds, increased with biodegradability.

In the presence of ultraviolet (UV) exposure, photo-transformation of 2,6-DNT began immediately after simulated solar radiation (SSR) in sandy and fine-grained sediment, with 89% being photo-transferred after 24 hours, and none remaining for photo-transfer after 72 hours (Nipper 2004).

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to DNT depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of DNT in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on DNT levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring DNT in a variety of environmental media are detailed in Chapter 7.

6.4.1 Air

Measurements of DNT in ambient air were not located. In an occupational environment, NIOSH (1980) measured breathing zone air concentrations of Tg-DNT that ranged from undetected to 23 µg/m³ (TWA). Tg-DNT concentrations in the area air samples ranged from undetected to 420 µg/m³ (TWA). NIOSH (1982) reported that area air samples in a manufacturing facility contained TWA concentrations of Tg-DNT that ranged from undetected to 890 µg/m³. Levine et al. (1985b) also measured personal air samples of 2,4-DNT at 10–440 µg/m³ and 2,4- and 2,6-DNT at 50–590 µg/m³ in the workplace.

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6.4.2 Water

DNT is rarely detected in public drinking water supplies. 2,4-DNT and 2,6-DNT are priority pollutants, and amendments to the Safe Drinking Water Act require that they be monitored for by large community water systems (CWSs) and non-transient non-community water systems (NTNCWSs) and a statistically representative sample of qualifying small CWSs and NTNCWSs (EPA 2008a, 2008b). Contaminant occurrence data collected under the First Unregulated Contaminant Monitoring Regulation (UCMR 1) showed only a single detection of DNT. Since the health reference level (0.05 µg/L) for each isomer was below the minimum reporting level of 2 µg/L, the data were reported as only as detections exceeding this level. Among the small public water systems (797 tested), there were no detections of 2,4-DNT in 3,251 samples taken, and there was only 1 detection out of 30,513 samples obtained from large systems throughout the United States (3,076 tested). This single detection of 333 µg/L was in a surface water sample obtained from an entry point source at a large public water system in the State of Tennessee (EPA 2008b). 2,6-DNT was not detected in any of the 33,765 samples (both large and small systems) for which it was tested. A total of 3,873 public water systems were tested for 2,6-DNT, of which 1,970 relied on ground water sources and 1,903 relied on surface water sources (EPA 2008b).

DNT has been detected in surface water and groundwater near source locations such as munitions sites. Concentrations of 2,4- and 2,6-DNT obtained from a small brook and the Losse River, Germany adjacent to a former ammunition plant were 0.5–13.0 and 0.1–7.6 µg/L, respectively (Feltes et al. 1990). Two ponds located on a closed munition site in Germany had concentrations of 2,4-DNT ranging from 0.8 to 1.2 µg/L and concentrations of 2,6-DNT ranging from 0.07 to 0.3 µg/L (Feltes et al. 1990). Monitoring studies at three polluted locations of the Elbe River in Germany found concentrations of 2,4-DNT ranging from 0.1 to 1.3 µg/L, while concentrations of 2,6-DNT ranged from 0.08 to 0.5 µg/L (Feltes et al. 1990). Sohr et al. (1995) reported 2,4- and 2,6-DNT concentrations of 0.7 and 3.1 µg/L, respectively, at contaminated warfare sites in Germany. Other less-contaminated sites contained 28 ng/L 2,4-DNT and 19 ng/L 2,6-DNT (Sohr et al. 1995). In the seawater of Dokai Bay in Japan, 2,4- and 2,6-DNT were detected at levels of not detected (ND)–206 and ND–14.8 µg/L, respectively, in an area receiving effluent from an industrial plant (Hashimoto et al. 1982). 2,3-DNT was also detected, but at much lower levels (ND–0.412 µg/L). DNT levels as high as 10,000 µg/L were reported in potable groundwater at the Joliet Army Ammunition Plant located in Will County, Illinois (EPA 2008a). 2,3-, 2,5-, 3,4-, and 3,5-DNT isomers were identified in both monitoring wells and a few private water supply wells near the Badger Army Ammunition Plant site in Wisconsin (EPA 2008a).

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6.4.3 Sediment and Soil

Hoke et al. (1993) reported that only low concentrations of 2,4- and 2,6-DNT were detected in sediment of the Great Calumet River-Indian Harbor. Concentrations of 2,4-DNT in sediment pore water ranged from the detection limit of 0.01 to 0.07 µg/L. 2,4-DNT concentrations in sediment pore water ranged from 0.1 to 1.7 µg/L. 2,6-DNT was not detected in sediment samples (limit of detection [LOD]=0.01 µg/L) and subsequently was not analyzed for in sediment pore water. 2,4-DNT and 2,6-DNT were identified in bed sediment collected from 20 major river basins in the United States for the 1992–1995 sampling period (Lopes and Furlong 2001). The maximum detected concentrations were reported as 170 µg/kg for 2,4-DNT (detected in 0.6% of 519 sites sampled) and 93 µg/kg (detected in 1% of 518 sites sampled) for 2,6-DNT.

Concentrations ranging from <0.1 mg/kg (detection limit) to 117 mg/kg of 2,4-DNT were found at the Joliet Army Ammunition Plant, in Joliet, Illinois, an NPL site. 2,6-DNT was detected on this site at concentrations ranging from <0.1 to 8 mg/kg (Simini et al. 1995). The concentrations of 2,4- and 2,6-DNT were reported as 19 and 1.38 g/kg, respectively, in soil samples obtained at the Volunteer Army Ammunition Plant located in Chattanooga, Tennessee and levels of 2,4- and 2,6-DNT were reported as 8.9 and 0.48 g/kg, respectively, in soil samples obtained at the Badger Army Ammunition Plant located in Wisconsin (UNEP 2004). The concentration of 2,4-DNT in soil samples at the Gyttrorp facility in Sweden, which was used for explosives manufacturing from 1864 to 1995 was 4 g/kg (UNEP 2004). The concentrations of 2,4- and 2,6-DNT measured in soil samples obtained from the decommissioned TNT manufacturing facility in Hessisch Lichtenau Germany were reported as 3.6 and 2.5 g/kg, respectively (UNEP 2004).

Residues of DNT were detected on the surface of military live-fire training soil (Jenkins 2006). Mean surface soil concentrations ranged from <0.001 to 84 mg/kg for 2,4-DNT and from <0.001 to 4.6 mg/kg for 2,6-DNT at 23 military firing ranges in the United States and Canada (Jenkins 2006).

6.4.4 Other Environmental Media

Neither 2,4- nor 2,6-DNT were detected in samples of fish obtained from Lake Michigan tributaries and Grand Traverse Bay (Camanzo et al. 1987). DNT was monitored for, but was not detected in, fish from Great Lakes harbors and tributaries in Ohio and Wisconsin (DeVault 1985).

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6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Sources of exposure to DNT for the general population include processing facilities that manufacture or process DNT, as well as hazardous waste sites that release this chemical. Populations residing near ammunition or explosive manufacturing facilities may be exposed to contaminated groundwater or soil. Exposure pathways to DNT include dermal contact and incidental ingestion pathways. Since DNT is not frequently detected in air, inhalation exposure to the general population is expected to be low.

Occupational exposure to DNT may occur from its use in the manufacture of toluene diisocyanate, in the production of explosives, in the manufacture of azo dye intermediates, and in organic synthesis in the preparation of toluidines and dyes (IARC 1996). Exposure may also occur at facilities that store or dispose the substance. Occupational exposure will involve inhalation, dermal contact, and incidental ingestion, with inhalation the most likely exposure pathway.

Studies on occupational exposure to DNT are limited. Levine et al. (1985b) evaluated the 7-hour TWA personal exposure of workers to Tg-DNT and measured urinary metabolites of DNT at a DNT manufacturing plant. Breathing zone exposure level of production unit operators to both 2,4- and 2,6-DNT averaged 0.26 mg/m^3 . Air exposure concentrations of loaders, who load storage tanks, collect samples, and perform cleaning tasks, averaged 0.32 mg/m^3 . Exposure of maintenance mechanics averaged 0.12 mg/m^3 , and the exposure of acid-stripper operators was 0.06 mg/m^3 . The highest personal air monitoring concentrations and levels of urinary metabolites were found to be for loaders, followed by process operators. The levels of urinary metabolites of DNT in loaders and operators exceeded those that would have resulted from the inhaled concentrations, although the workers wore gloves for operations that might have led to dermal exposure. Woollen et al. (1985) carried out biological monitoring studies of 28 workers at an explosives factory. The 2,4-DNT metabolite, 2,4-dinitrobenzoic acid was not detected or was present at very low levels in urine samples prior to the start of work; however, it was detected in all post-shift urine samples. The weakly mean post-shift urine concentration of 2,4-dinitrobenzoic acid was 17 mg/L . Concentrations of 2,4-DNT in air samples of the plant ranged from 0.02 to 2.68 mg/m^3 . It was concluded that air concentrations could not solely account for the observed excretion levels of the metabolite, 2,4-dinitrobenzoic acid, indicating probable dermal uptake or inadvertent ingestion.

A cross-sectional study with 82 employees who dismantled military waste at the mechanical plant in Saxony, Germany was undertaken by Letzel et al. (2003). The maximum concentrations of 2,4-DNT in the ambient air was $20 \text{ } \mu\text{g/m}^3$. The maximum concentrations in the urine of workers regularly exposed to

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the ammunition were 2.1 µg/L for 2,4-DNT, 95 µg/L for 2,4-dinitrobenzoic acid, and 3.6 µg/L for 2,6-DNT.

In another study, Levine et al. (1986a) reported increases of heart disease among workers at two ammunition plants; one of which is located in Joliet, Illinois and the other located in Radford, Virginia. DNT was manufactured and purified at the plant in Joliet, Illinois and was used in single-base propellant formulations at the plant in Radford, Virginia.

OSHA established an 8-hour TWA Permissible Exposure Limit (PEL) for DNT as 1.5 mg/m³, with skin designation to indicate the potential significant contribution to the overall exposure by the cutaneous route (OSHA 2010). The American Conference of Governmental Industrial Hygienist (ACGIH)'s Threshold Limit Value (TLV) for DNT is 0.2 mg/m³, with skin notation (ACGIH 2011). TLV is the time-weighted average concentration for a conventional 8-hour workday and a 40-hour workweek to which it is believed that nearly all workers may be repeatedly exposed without adverse effect. The National Institute of Occupational Safety and Health (NIOSH) determined the Recommended Exposure Limit (REL) for DNT as 1.5 mg/m³, with skin designation (NIOSH 2011).

6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

No studies are available that monitor the level of exposure of children to DNT. No measurements have been made of DNT or its metabolite levels in amniotic fluid, meconium, cord blood, or neonatal blood to test for prenatal exposure, nor have measurements been made of DNT or metabolite levels in breast milk.

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However, because of the low octanol-water partition coefficient of DNT and excretion in the urine, it is not expected to accumulate in maternal tissues.

Although DNT can degrade in the environment, it has been detected at high levels at artillery sites or ammunition producing facilities. Therefore, children playing in soil contaminated with DNT have the potential to be more exposed than adults, both because of this behavior and because of their larger skin surface area in proportion to their body weight for dermal absorption. Also, children drinking well water contaminated with DNT might be exposed to more of the chemical than adults would be due to the fact that children drink more fluids per kilogram of body weight than adults. Significant dietary exposure is unlikely as DNT is not expected to accumulate in animal tissues. However, ingestion of vegetables and crops grown in DNT contaminated areas could be a source of exposure.

There were no studies that examine potential exposure of children from their parents work clothes, skin, hair, tools, or other objects removed from the workplace. No information is available concerning exposure from consumer products because DNT is used mainly for military and industrial purposes.

Although DNT is genotoxic in *in vivo* test systems, it is found to be negative in dominant lethal mutations (U.S. Army 1979) and spermatocyte DNA repair (Working and Butterworth 1984). There is no evidence that exposure of parental germ cells to the active form of DNT could plausibly occur since DNT does not accumulate in tissue.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

In addition to individuals who are occupationally exposed to DNT (see Section 6.5), there may be groups within the general population that have potentially high exposures (higher than background levels) to DNT. These populations include individuals living in proximity to sites where DNT was produced or sites where DNT was disposed, and individuals living near one of the NPL hazardous waste sites where isomers of DNT have been detected in some environmental media (HazDat 2007).

Based on the available information, it appears that the highly-exposed populations would be workers exposed in manufacturing facilities.

Members of the general population are likely to be exposed only if they are near a local source of contamination, such as an industrial discharge or an abandoned waste site. There is also the possibility of

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exposure to DNT from the gradual turnover of former military bases to local communities for public use. DNT does not appear to be widespread in the environment, and they were not frequently detected at hazardous waste sites.

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of DNT 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 DNT.

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

6.8.1 Identification of Data Needs

Physical and Chemical Properties. Information regarding the physical and chemical properties of a chemical is essential for estimating the partitioning of the chemical in the environment. Information on the physical and chemical properties of DNT is presented in Chapter 4 and the data appear to be adequate (HSDB 2012). The isomers of DNT have many similar traits, including identical molecular weights, but 2,4-DNT has higher melting and boiling points and a greater solubility in water than 2,6-DNT (HSDB 2012). DNT is generally produced as a technical-grade mixture comprised of 95% 2,4- and 2,6-DNT and 5% other substances. The other substances are predominantly isomers of DNT not discussed in this profile.

Production, Import/Export, Use, Release, and 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 substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2012, became available in 2010. This database is updated yearly and should provide a list of industrial production facilities and emissions.

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USITC statistics on synthetic organic chemical production (USITC 1987) do not describe DNT production. Uses of DNT appear to be well characterized (CMR 1983; HSDB 2012). The most commercially important use of DNT is as a chemical intermediate in the production of toluene diisocyanate, a precursor to polyurethane polymers (HSDB 2012). It has been estimated that 99% of all DNT produced is used for this purpose (CMR 1983). DNT is recognized as a potentially hazardous chemical and is subject to a variety of regulations (see Chapter 8), but disposal practices and restrictions are not adequately documented.

Environmental Fate. The low octanol-water partition coefficients of the DNT isomers predict that DNT released to the environment would not bioaccumulate and would be weakly bound to soil organic matter. Bioconcentration data also supports the notion that 2,4-DNT is not bioaccumulative in fish (NITE 2002; Lang 1997). The relatively low volatility and high solubility of DNT indicate that it will tend to remain in water for long periods of time unless acted upon by light, oxygen, or biota, creating the potential for transportation to groundwater or surface water (Jenkins et al. 1986). DNT has been found in waste water and groundwater in and around munitions sites (Jenkins et al. 1986; Spanggord and Suta 1982; UNEP 2004). The occurrence of DNT in waste water from other manufacturing uses such as polyurethane forms has not been reported.

Given the importance of information about the behavior of DNT in the water column, and the extensive range of available information relative to that topic (EPA 1982c; Gillett 1983; Hashimoto et al. 1982, 1984; Jenkins et al. 1986; U.S. Army 1980), the absence of substantive information about DNT releases to, or fate in, soils and air is less troublesome than it might be for many chemicals. Data on the persistence of DNT in the vadose zone (the unsaturated zone lying between the ground level and the top of the groundwater) and groundwater are needed, as well as measured rates of plant uptake and metabolism. Because of the structurally specific nature of biotransformations, more information on the fate of DNT metabolites would be welcome.

Bioavailability from Environmental Media. No information is currently available that describes the bioavailability of DNT in food. Data on bioavailability of soil/sediment residues would be helpful. Neither 2,4- nor 2,6-DNT were detected in samples of fish obtained from Lake Michigan tributaries and Grand Traverse Bay (Camanzo et al. 1987). DNT was not detected in fish from Great Lakes harbors and tributaries in Ohio and Wisconsin (DeVault 1985).

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Food Chain Bioaccumulation. Limited information indicates that DNT is not widely distributed in the environment. Residues of DNT have a low frequency of detection in water samples. These data indicate that bioaccumulation may not be an area of concern (Callahan et al. 1979; EPA 1982c). The log K_{ow} values for 2,4- and 2,6-DNT are 1.98 and 2.10, respectively (Callahan et al. 1979; EPA 1982c), indicating that bioaccumulation is not likely to occur. BCFs also indicate that DNT is not expected to bioaccumulate in fish (NITE 2002; Lang 1997). Degradation of DNT forms a variety of products, about which very little is known. Additional information would help to confirm or refute indications of low potential for bioaccumulation of DNT in foods.

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

The sparse information base suggests that widespread contamination by DNT has not occurred (Staples et al. 1985). DNT was detected at very low frequencies in drinking water (EPA 2008b). Analyses of wastewaters indicate that local contamination may occur (Feltz et al. 1990; Shackelford and Keith 1976; Spanggord and Suta 1982; Spanggord et al. 1982a). DNT was also present in the sediment and soil near source areas (Hoke 1993; Simini 1995; Jenkins 2006).

Exposure Levels in Humans. This information is necessary for assessing the need to conduct health studies on these populations. No studies of exposure of the general population were found, and the occupational studies (Levine et al. 1985b; Woollen et al. 1985) are inadequate to ascertain "background" or nonoccupational exposure. Based on available information, the highly-exposed populations are those workers exposed in manufacturing facilities. Members of the general population are likely to be exposed only in that they are near a local source of contamination. Toxicokinetic data on occupationally- and environmentally-exposed humans will be helpful. Measurements of DNT and its metabolite levels in blood and urine will be useful to provide an estimate of internal dose of exposure.

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. No exposure or body burden studies have been conducted on children; consequently, it is not known if children differ from adults in their weight-adjusted intake of DNT, or if unique exposure pathways for children exist. Since DNT is not a widespread environmental contaminant,

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there are only two likely potential sources of exposure for children. Children living near a DNT contaminated site might be exposed if DNT has moved offsite in contaminated environmental media. If such a situation were identified, further site-specific studies of children's exposure could be conducted. Children whose parents work in manufacturing facilities that produce or use DNT and are occupationally exposed to significant quantities of DNT might potentially be exposed to DNT transported home on their parents' work clothes, skin, hair, tools, or other objects removed from the workplace. If such a significant occupational exposure setting were identified, they might be the subject of a take-home exposure study.

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

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

The development of an exposure registry would provide valuable data on exposure levels and frequency. In addition to providing information on exposure levels and duration, a registry would be useful in identifying sources of exposure such as hazardous waste sites and manufacturing and use facilities. Knowledge about exposure levels and sources would be valuable in developing strategies to control unnecessary sources and these exposures. The ability to correlate sources and exposure levels with health effects would be useful in identifying disease conditions that may result from exposure to the chemical.

6.8.2 Ongoing Studies

No ongoing studies sponsored by NIH or EPA were identified for DNTs.

7. ANALYTICAL METHODS

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

7.1 BIOLOGICAL MATERIALS

The need to determine DNT in biological materials could arise from occupational exposure in the manufacture and processing of 2,4,6-TNT and from exposure to waste water and waste disposal sites associated with TNT manufacture. It has been noted (Jenkins et al. 1986) that "one of the Army's most serious water pollution problems is the disposal of wash waters used to clean equipment and interior surfaces at munitions manufacturing and demilitarization facilities." The same reference mentions the generation of large quantities of waste water from these facilities.

Although there are numerous occupational monitoring studies, a limited number of methods regarding the determination of DNT in biological samples are available in the literature. DNT has been determined in ocean floor fauna using thin layer chromatography (TLC) (U.S. Navy 1972). Procedures have been described for the examination of swabs for traces of explosives, including 2,6-DNT using high-performance liquid chromatography (HPLC) with electrochemical detection at a pendant drop electrode (Lloyd 1983a). These techniques can be applied to biological materials such as skin surfaces exposed to explosives. DNT and its metabolites were determined in blood and urine by gas chromatography (GC) techniques (Turner et al. 1985; Woollen et al. 1985) and in urine by TLC (Woollen et al. 1985). Qualitative determination of DNT and its metabolites can also be performed after reduction to primary arylamines and subsequent coupling of diazo compounds to produce a colored complex, which absorbs light at 550 nm (Smith et al. 1995).

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Dichloromethane is the solvent of choice for extracting DNT from water samples (EPA 1982a) and from wastes (EPA 1986a). Reversed-phase high-performance liquid chromatography (RP-HPLC) is attractive for the determination of DNT in waste water because it enables direct analysis of aqueous samples (Jenkins et al. 1986). A medium similar to the mobile phase used in this HPLC separation, i.e., 50/38/12 (v/v/v) water/methanol/acetonitrile, should be suitable for extracting DNT from low-lipid biological samples and for subsequent HPLC determination after sample cleanup.

In a study conducted by Honeychurch et al. (2003), screen-printed carbon electrodes (SPCEs) were used as disposable sensors for the measurement of 2,6-DNT using a stripping voltammetric method. Voltammograms showed the appearance of two reduction peaks that corresponded to the formation of the hydroxylamine derivatives from the reduction of the nitro groups and one oxidation peak possibly from the formation of the nitrosamines. The detection limit was 161 ng/mL and the sensors were evaluated by determining concentrations of 2,6-DNT in spiked and unspiked potable water, saliva, and dust wipes. Percent recoveries for spiked samples were 92.8–113% for potable water, 41.0–52.0% for saliva, and 67.6–79.1% for dust wipes. 2,6-DNT was not detected in unspiked samples. This new study indicates that SPCEs could possibly be used in the analytical detection of DNT in occupational settings.

Methods for the determination of the DNT in biological samples are given in [Table 7-1](#).

7.2 ENVIRONMENTAL SAMPLES

The basic method for collecting DNT from the ambient atmosphere is adsorption on a solid phase, such as granular adsorbents (silica gel), filters, and impingers, followed by removal with solvents such as chloroform. Bubbler collectors can also be used for direct collection of analyte in a non-volatile solvent such as ethylene glycol. New instrumentation for the detection of 2,4-DNT and other explosives has recently been developed that will accept both air and surface particulate samples (Nacson et al. 1994). The instruments consist of capillary GC columns terminating in an electron capture detector (ECD); the detection limit for 2,4-DNT is 20 ppt (Nacson et al. 1994). Also available is a portable version that is useful for a wide variety of applications, such as security checks, mail or passports, or in high-risk facilities (Nacson et al. 1994).

Sylvia et al. (2000) developed a Surface-Enhanced Raman Spectroscopy (SERS) to potentially detect 2,4-DNT vapors above TNT-based landmines. This method of detection exhibited reproducible results, sensitive detection levels of ≤ 5 ppb 2,4-DNT, and spectra that demonstrated high signal-to-noise ratios

7. ANALYTICAL METHODS

Table 7-1. Analytical Methods for Determining DNT in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine containing DNT and metabolites	Hydrolysis of metabolites, extraction, derivatization	GC/MS	0.1 mg/L	NR	Turner et al. 1985
Urine containing DNT and metabolites	Zinc-catalyzed reduction of DNT with hydrochloric acid to primary arylamines; diazotize and couple with <i>N</i> -(1-naphthyl)ethylene diamine to produce a colored complex with characteristic adsorption at 550 nm	UV/VIS	100 ng/mL	NR	Smith et al. 1995
Urine containing metabolites	Extraction with ethyl acetate	GC	0.1 mg/L	NR	Woollen et al. 1985
Urine	Extraction with ethyl acetate	TLC	0.1 mg/L ^a	NR	Woollen et al. 1985
Plant tissue	Extract with sonication; cleanup on flonsil; alumina (LC); inject	Reverse-phase LC UV/VIS	0.02–10 mg/mL	–	U.S. Army 1998
Skin	Swab with ethanol	HPLC/ED	5.6 ng/mL ^a	97 (2,6-DNT); 93 (2,4-DNT)	Lloyd 1983a
Blood	Extraction with toluene	GC	0.00001 mg/mL ^a		Woollen et al. 1985
Ocean floor fauna	NR	TLC	NR		U.S. Navy 1972
Skin	Swab with ethanol	HPLC/ED	5.6 ng/mL ^a	97 (2,6-DNT); 93 (2,4-DNT)	Lloyd 1983a

^aLowest detected concentration.

DNT = dinitrotoluene; ECD = electron capture detection; ED = electrochemical detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; LC = liquid chromatography; MS = mass spectrometry; NR = not reported; TLC = thin layer chromatography; UV/VIS = ultra-violet/visible spectroscopy

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collected in 30 seconds. While only preliminary blind testing was done, vapor-phase SERS is a promising instrumental method for the detection of explosive vapors.

Albert and Walt (2000) developed a novel cross-reactive optical microsensor for high-speed detection of low-level explosives and explosive-like vapors. This porous silica-based sensor, was used to detect DNT in water vapor at a level of 23 ppb in clean dry air.

Likewise, a field developed instrument with artificial cross-reactive optical sensors was employed for the detection of 2,4-DNT vapors in spiked soil and aqueous and ground samples. Two types of fluorescence based vapor sensors were developed: one sensor was semiselective for the nitroaromatic compounds (NAC), while the other was non-specific and cross reactive. The fluorescent sensors were exposed to multiple solvents and were validated/calibrated by making measurements of known and estimated concentrations of 2,4-DNT. The system has demonstrated the ability to detect 120 ppb 2,4-DNT vapor in blind humidified samples and has been exclusively used in field tests (Albert et al. 2001).

DNT is most commonly extracted with dichloromethane from water samples (EPA 1982a) and from wastes (EPA 1986a). A continuous countercurrent liquid-liquid extraction method is useful in extracting DNT from surface water samples (Deroux et al. 1996). The advantage of this method is that it is capable of extractions from large sample volumes and unfiltered natural water samples (Deroux et al. 1996). A sonic extraction-liquid chromatographic method has been used for detection of 2,4-DNT in soils (Bauer et al. 1990; Griest et al. 1993). A simple screening method has been developed for the detection of 2,4-DNT in field soil samples that utilizes the spectrophotometer for identification by colorometrics after an initial reaction of the extract with potassium hydroxide and sodium sulfite (Jenkins and Walsh 1992).

A biosensor has been developed by Smirnova et al. (2004) for the detection of the dinitrotoluenes in soil and groundwater. The genetic system from the *Burkholderia sp.* strain DNT was examined and used to develop the prototype cell-based biosensor. The central element of the biosensor was found to be two crystal structures of the transcriptional regulator DntR with acetate and thiocyanate occupying the inductor-binding cavity. Analysis of this biosensor was done to model how 2,4-DNT might bind to DntR and to ultimately make conclusions on how the specificity of the biosensor might be enhanced.

The analysis of DNT is normally done by GC with a variety of detectors, including flame ionization detector (FID), electron capture detection (ECD), Hall electrolytic conductivity detector (HECD), thermionic specific detector (TSD), fourier transform infrared (FT-IR), thermal energy analyzer (TEA), or

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mass spectrometry (MS). It has been noted (EPA 1986b) that 2,4-DNT is "subject to erratic (gas) chromatographic behavior." When mass spectrometry is used to analyze water samples for DNT, electron impact (EI) is preferentially used because many structure-specific fragments will be formed, which can be used for identification of isomers (Feltz et al. 1990). To improve the accuracy of mass spectroscopic techniques in the identification of pollutants in aqueous and solid matrices, EPA has developed the method of isotope dilution (EPA 1989). Isotope dilution employs stable, isotopically labeled analogs of both 2,4- and 2,6-DNT to be used as internal standards in GC/MS analysis (EPA 1989). Negative-ion chemical ionization has been shown to have a higher sensitivity and selectivity than EI, however, and should be used when determining traces of nitroaromatic compounds in complex aqueous mixtures (Feltz et al. 1990).

The quenching of the photoluminescence of porous silicon films in a flowing air stream once the films were exposed to DNT was monitored and the quantity of DNT was detected in a study by Content et al. (2000). This detection was achieved by catalytic oxidation of DNT. The detection limit of 2 ppb was observed for 2,4-DNT.

TLC and high-performance thin layer chromatography (HPTLC) have also been used to identify and quantify 2,4- and 2,6-DNT in soil and water samples from contaminated waste sites (Griest et al. 1993; Sohr et al. 1995; Steuckart et al. 1994). GC analysis is difficult because of the large amounts of humic acids present which cause overlap of matrix signals without cleanup; therefore, HPTLC can be a more advantageous method (Steuckart et al. 1994). Cleanup is not necessary with HPTLC, except in the analysis of soil samples (Steuckart et al. 1994).

A sensitive method for the analysis of DNT in drinking water has been developed using wide-bore fused silica capillary column GC with an ECD (Hable et al. 1991). The detection limits of this method are 0.04 µg/L for 2,4-DNT and 0.003 µg/L for 2,6-DNT; these detection limits are sensitive enough to meet the suggested requirements for EPA health advisories and water quality criteria.

Also, HPLC with UV-detection for nitroaromatic compounds and amperometric detection for aminoaromatic and phenolic compounds were used by Spiegel (1997) for the monitoring of the degradation products of explosives in groundwater. These methods of detection were reported to be extremely sensitive. 2,4-DNT and 2,6-DNT were observed to be nearly completely metabolized as seen by their decrease in the chromatograms.

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For the determination of 2,4-DNT in munitions manufacture waste water, RP-HPLC was chosen by Jenkins et al. (1986) because it enables direct analysis of samples in aqueous solution without prior extraction, attains adequate detection limits without preconcentration, and avoids problems with analyte thermal instability. The detection limit for 2,4-DNT was 10 µg/L with a standard deviation of 3.4 µg/L for concentrations up to 250 µg/L. A convenient method for analysis of 2,4- and 2,6-DNT in contaminated soils used high performance liquid chromatography with minimal sample preparation (Preslan et al. 1993). Likewise, DNT was also measured in field soil samples by using a method that included the fortification of DNT in the soil, recovery of DNT by pressurized fluid extraction, and measurement of DNT by gas and liquid chromatography-MS (Campbell et al. (2003)). Compared with the former EPA method SW-846 8330, this method provided a quicker time frame needed to complete an extraction (15 minutes as opposed to 18 hours) and MS confirmation of the analytes. The limit of quantitation for 2,4-DNT was 0.05 µg/g.

Methods for the determination of DNT in environmental samples are summarized in [Table 7-2](#).

7.3 ADEQUACY OF THE DATABASE

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

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

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. The available methods for the determination of DNT and its metabolites in biological samples are inadequate. Although one method exists for determination of DNT and its metabolites in urine (Smith et al. 1995; Turner et al. 1985;

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Silica gel, desorb with chloroform	GC	0.1 mg/m ³	70–84	Hunt et al. 1980
Air	Dispersed on glass beads, glass tube, and Flowmeter/mixer; exposure to dry synthetic air	FT-IR/MCT detector, NMR	2 ppb (2,4-DNT)		Content et al. 2000
Air	Collect sample and insert into analyzer	GC/ECD	20 ppt (2,4-DNT)		Nacson et al. 1994
Water	Extraction with dichloromethane	GC/ECD or TEA	ECD: 3.8x10 ⁻¹⁴ g/s (2,4-DNT); TEA: 1.77x10 ⁻¹¹ g/s (2,4-DNT)		Feltes et al. 1990
Water	Extraction with dichloromethane	GC/MS (electron impact–full scan)	47 pg (2,6-DNT)		Feltes et al. 1990
Water	Extraction with dichloromethane; add methanol	GC/MS	5 ppb		Yinon 1996
Water	Adjust pH of spiking solution to >11 with NaOH; extraction with dichloromethane; add anhydrous sodium sulfate; filter; rotary evaporate	Liquid-liquid extraction with GC/MS	0.8 µg/L (2,4-DNT); 1.4 µg/L (2,6-DNT)	96 (2,4-DNT); 100 (2,6-DNT)	Yook et al. 1994
Water	Counter current liquid-liquid extraction method	GC/MS (EI and PICI)	2 ng/L	89	Deroux et al. 1996
Water	Adjust to pH 12 and pump into column; remove organic phase; add to retinate and adjust to pH 2 before pumping into column	GC/MS	2 ng/L	89	Deroux et al. 1996
Water	Spike H ₂ O sample with standards; conduct SPE; elute and dry under nitrogen	TLC (254 nm)	20 ng (scanner) 40 ng (visually)	diol-119-115.3 RP-18-100.6-102	Kessel and Hauck 1996

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water	Extraction with 0.5 mL toluene; rotate 30 minutes at 15 RPM	GC/ECD	0.003 µg/L (2,6-DNT); 0.04 µg/L (2,4-DNT)	93–103 (2,6-DNT); 93–96 (2,4-DNT)	Hable et al. 1991
Groundwater and surface water	Extraction with dichloromethane; acidify water sample with HCl and extract with isobutyl-methyl ketone; concentrate in rotary evaporator; dissolve residues in dichloromethane	TLC	10–20 ng/spot		Sohr et al. 1995
Groundwater	Extraction with dichloromethane	HPTLC-AMD	20 ng (2,4- and 2,6-DNT)		Steukart et al. 1994
Groundwater	Expose three samples to different amounts of sunlight; inject sample, loop	HPLC/UV (245 nm)	0.01–0.1 µg/L	–	Spiegel and Welsch 1997
Waste water (for 2,4-DNT)	Diluted directly with methanol and acetonitrile	HPLC/UV	4.6 µg/L	NR	Jenkins et al. 1986
Waste water	Extraction with dichloromethane	GC/IDMS	10 µg/L	10 (2,4-DNT); 17 (2,6-DNT)	EPA 1980a
Waste water	Extraction with dichloromethane, exchange to hexane	GC/ECD	NR		EPA 1982a
Waste water	Extraction with dichloromethane; EPA Method 8090	GC/MS	5.7 µg/L (2,4-DNT); 1.9 µg/L (2,6-DNT)		EPA 1986a
Non-water miscible waste	Extraction with dichloromethane; EPA Method 8090	GC/ECD	2,000 µg/L (2,4-DNT); 1,000 µg/L (2,6-DNT)		EPA 1986a
Biosludge	Extraction with sulfuric acid and dichloromethane	GC/TEA	0.05 mg/L	84	Phillips et al. 1983
Soil	Extraction with acetonitrile in ultrasonic bath; flocculate supernatant with CaCl ₂ ; filter	SE/LC		95–97	Bauer et al. 1990
Soil	Dilution directly with methanol	HPLC/PDA	40–80 pg/µL		Emmrich et al. 1993

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Extraction with acetone; filter supernatant; react with potassium hydroxide and sodium sulfite	Spectro-photometry	2 µg/g (2,4-DNT)		Jenkins and Walsh 1992
Soil	Grind soil; extraction with acetone in ultrasonic bath; centrifuge; add 5 mL toluene and remove acetone; dry toluene extract over anhydrous sodium sulfate	HPTLC/AMD	20 ng (2,4- and 2,6-DNT)		Steuckart et al. 1994
Soil	Fortification, extraction	HPLC-DAD, LC/MS, GC/MS	0.05 µg/g		Campbell et al. 2003
Soil, sediment, solid waste	Extraction	GC/MS	660 µg/kg		EPA 1986b
Soil, sediment, solid waste	Extraction with dichloromethane	GC/FT-IR	10 µg/L		EPA 1986c
Soil, sediment, solid waste	Extraction	GC/FT-IR	10 µg/L	NR	Gurka et al. 1987
Soil, water, and municipal sludges	Extraction with dichloromethane; addition of isotopically labeled analog	GC/MS	10 µg/mL	NR	EPA 1989
Soil/compost	Acid leaching followed by sonic extraction	HPLC	0.055–0.248 mg/L ^c	NR	Griest et al. 1993
Soil/compost	Extraction with acetonitrile; combine with CaCl ₂ ; derivatize with TFAA, then deactivate with H ₂ O	HPLC	–	NR	Preslan et al. 1993
Materials exposed to DNT (bomb debris)	Swab with ethanol	HPLC/ED	5.6 µg/L	97 (2,6-DNT); 93 (2,4-DNT)	Lloyd 1983b
Materials exposed to DNT	Swab surface and insert into outlet port	GC/ECD	20 ppt (2,4-DNT)		Nacson et al. 1994
Phenolic and nitroaromatic compounds	Inject sample, loop-separate using supercritical CO ₂ as mobile phase	SFC (230 nm)	oxidative-250 pg – reductive-100 pg		Wallenborg et al. 1997

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Explosives	Extract in acetonitrile and dilute in pH 7 buffer; inject hydrostatistically and detect by UV	MECC (214 nm)	0.55–0.74 mg/L	–	Oehrle 1996

^aAnalyses for both 2,4-DNT and 2,6-DNT unless otherwise noted.

^bMinimum detection to ECD.

^cVaried over course of experiment.

AMD = automated multiple development; CaCl₂ = calcium chloride; CO₂ = carbon dioxide; DAD = diode array detection; DNT = dinitrotoluene; ECD = electron-capture detection; ED = electrochemical detection; EI = electron ionization; FT-IR = fourier transform infrared; GC = gas chromatography; HCl = hydrochloric acid; HPLC = high-performance liquid chromatography; HPTLC = high-performance thin layer chromatography; IDMS = isotope dilution mass spectrometry; LC = liquid chromatography; MCT = mercury cadmium telluride; MECC = micellar electrokinetic capillary chromatography; MS = mass spectrometry; NaOH = sodium hydroxide; NMR = nuclear magnetic resonance; NR = not reported; PDA = photodiode array detection; PICl = positive ion chemical ionization; RPM = revolutions per minute; SE = solid extraction; SFC = super critical fluid chromatography; SFE = supercritical fluid extraction; SPE = solid-phase extraction; TDM = thermal desorption modulator interface; TEA = thermal energy analysis; TFAA = trifluoroacetic anhydride; TLC = thin layer chromatography; UV = ultraviolet absorption

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Woolen et al. 1985), blood (Woolen et al. 1985), and skin (Lloyd 1983a; Nacson et al. 1994), there is a need for modern validated standard methods of analysis for such data in plant and animal tissues and exudates. Methods do exist for water and waste water (EPA 1982a, 1982b) and for solid wastes (EPA 1986a, 1986b, 1986c). A method that employs the use of SPCEs has the potential to be useful for examining biological samples such as potable water, saliva, and dust wipes (Honeychurch et al. 2003). The need also exists for good methods to determine DNT biomarkers in biological materials. The determination of this compound in plant and animal tissues and exudates would be useful to help determine exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. DNT can be analyzed in water, air, and waste samples with reasonable selectivity and sensitivity (EPA 1986a, 1986b, 1986c, 1989; Gurka et al. 1987; Nacson et al. 1994; Yinon 1996; Yook et al. 1994). Therefore, there is a reasonable database in this area.

There exists an ongoing effort to develop a "Master Analytical Scheme" for organic compounds in water (Michael et al. 1988). The overall goal is to detect and measure quantitatively organic compounds at 0.1 µg/L in drinking water, 1 µg/L in surface waters, and 10 µg/L in effluent waters. Analytes include numerous semivolatile compounds and some compounds that are only "semi-soluble" in water, as well as volatile compounds (boiling point <150 °C). It may be anticipated that improved methods for the determination of semivolatile DNT isomers in environmental samples may be developed as part of this effort.

7.3.2 Ongoing Studies

No ongoing studies regarding analytical methods sponsored by NIH or EPA were identified for DNTs.

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8. REGULATIONS, ADVISORIES, AND GUIDELINES

MRLs are substance specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites.

ATSDR has derived an acute-duration oral MRL of 0.05 mg/kg/day for 2,4-DNT based on neurotoxicity in Beagle dogs exposed to 2,4-DNT for the first 14 days of a 13-week study (Ellis et al. 1985; U.S. Army 1978b). The MRL was derived using the NOAEL value of 5 mg/kg/day for neurotoxicity in dogs. An uncertainty factor of 100 was used (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR has derived an intermediate-duration oral MRL of 0.007 mg/kg/day for 2,4-DNT based on hematological effects in Beagle dogs exposed to 2,4-DNT for 9 months (Ellis et al. 1985; U.S. Army 1979). The MRL was derived using BMD modeling of data for decreased hematocrit in female dogs. The dose associated with a change of 1 standard deviation from controls (BMD_{1SD}) for decreased hematocrit was 2.19 mg/kg/day; the lower 95% confidence limit on this dose ($BMDL_{1SD}$) was 0.67 mg/kg/day. An uncertainty factor of 100 was used (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR has derived a chronic-duration oral MRL of 0.001 mg/kg/day for 2,4-DNT based on hematological effects in Beagle dogs exposed to 2,4-DNT for 12 months (Ellis et al. 1985; U.S. Army 1979). The MRL was derived using BMD modeling of data for decreased erythrocyte count in female dogs. The dose associated with a change of 1 standard deviation from controls (BMD_{1SD}) for decreased hematocrit was 0.35 mg/kg/day; the lower 95% confidence limit on this dose ($BMDL_{1SD}$) was 0.12 mg/kg/day. An uncertainty factor of 100 was used (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR has derived an acute-duration oral MRL of 0.09 mg/kg/day for 2,6-DNT based on hematological effects in Beagle dogs exposed to 2,6-DNT for the first 2 weeks of a 13-week study (U.S. Army 1976). The MRL was derived using BMD modeling of data for decreased erythrocyte count in dogs. The dose associated with a change of 1 standard deviation from controls (BMD_{1SD}) for decreased erythrocyte count was 12.21 mg/kg/day; the lower 95% confidence limit on this dose ($BMDL_{1SD}$) was 9.31 mg/kg/day. An

8. REGULATIONS, ADVISORIES, AND GUIDELINES

uncertainty factor of 100 was used (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR has derived an intermediate-duration oral MRL of 0.004 mg/kg/day for 2,6-DNT based on an increased incidence of mild extramedullary erythropoiesis in the spleens of Beagle dogs exposed to 2,4-DNT for the first 14 days of a 13-week study (U.S. Army 1976). The MRL was derived using the LOAEL value of 4 mg/kg/day for an increased incidence of extramedullary erythropoiesis in dogs. An uncertainty factor of 1,000 was used (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

The International Agency for Research on Cancer (IARC) classifies 2,4- and 2,6-DNT as Group 2B carcinogens (*possibly carcinogenic to humans*) and 3,5-DNT as a Group 3 carcinogen (*not classifiable as to its carcinogenicity to humans*) (IARC 2012). The U.S. EPA classifies the 2,4-/2,6-DNT mixture as B2 (*probable human carcinogen*) (IRIS 2012). The American Conference of Governmental Industrial Hygienists (ACGIH) has classified the 2,4-/2,6-DNT mixture as an A3 carcinogen (*confirmed animal carcinogen with unknown relevance to humans*) (ACGIH 2011).

OSHA requires employers of workers who are occupationally exposed to the 2,4-/2,6-DNT mixture to institute engineering controls and work practices to reduce and maintain employee exposure at or below permissible exposure limits (PEL) of 1.5 mg/m³ (OSHA 2012).

The EPA (IRIS 2012) has derived an oral reference dose (RfD) of 0.002 mg/kg/day for 2,4-DNT based on a NOAEL of 0.2 mg/kg/day for neurotoxicity, Heinz bodies, and biliary tract hyperplasia in Beagle dogs in a 2-year feeding study (Ellis et al. 1985). The EPA (IRIS 2012) also derived an oral slope factor (q1*) of 0.068 (mg/kg/day)⁻¹ based on multiple tumor types in female rats (U.S. Army 1979) for the 2,4-/2,6-DNT mixture.

2,4-/2,6-DNT mixture and 3,4-DNT are on the list of chemicals in “The Emergency Planning and Community Right-to-Know Act of 1986” (EPCRA) and have been assigned a reportable quantity (RQ) limit of 10 pounds combined (EPA 2011). 2,4-DNT, and 2,6-DNT also appear on the EPCRA list of chemicals and has been assigned a reportable quantity (RQ) limit of 10 and 100 pounds, respectively (EPA 2011).

8. REGULATIONS, ADVISORIES, AND GUIDELINES

The 2,4-/2,6-DNT mixture is designated as a hazardous substances under Section 311 of the Clean Water Act; any discharge of these chemicals over a specified threshold level (10 pounds) into navigable waters is subject to reporting requirements (EPA 2011k).

The international and national regulations, advisories, and guidelines regarding 2,4- and 2,6-DNT in air, water, and other media are summarized in [Table 8-1](#).

8. REGULATIONS, ADVISORIES, AND GUIDELINES

Table 8-1. Regulations, Advisories, and Guidelines Applicable to DNT

Agency	Description	Information	Reference
<u>INTERNATIONAL</u>			
Guidelines:			
IARC	Carcinogenicity classification		IARC 2012
	2,4-DNT	Group 2B ^a	
	2,6-DNT	Group 2B ^a	
	3,5-DNT	Group 3 ^b	
WHO	Air quality guidelines	No data	WHO 2010
	Drinking water quality guidelines	No data	WHO 2008
<u>NATIONAL</u>			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV (8-hour TWA)		ACGIH 2011
	DNT ^{c,d}	0.2 mg/m ³	
AIHA	ERPGs	No data	AIHA 2011
DOE	PAC-1 ^e		DOE 2012
	DNT, 2,4-, 2,6-, and 3,4-DNT	0.6 mg/m ³	
	PAC-2 ^e		
	DNT	1.1 mg/m ³	
	2,4-DNT	4.9 mg/m ³	
	2,6-DNT	12 mg/m ³	
	3,4-DNT	33 mg/m ³	
	PAC-3 ^e		
	DNT, 2,4-, 2,6-, and 3,4-DNT	2,000 mg/m ³	
EPA	AEGIs	No data	EPA 2011b
	Hazardous air pollutant	No data	EPA 2012b 42 USC 7412
	NAAQS	No data	EPA 2010
NIOSH	REL (10-hour TWA)		NIOSH 2011
	DNT ^{c,f,g}	1.5 mg/m ³	
	IDLH		
	DNT ^{c,f,g}	50 mg/m ³	
OSHA	PEL (8-hour TWA) for general industry		OSHA 2012
	DNT ^{c,g}	1.5 mg/m ³	29 CFR 1910.1000, Table Z-1

8. REGULATIONS, ADVISORIES, AND GUIDELINES

Table 8-1. Regulations, Advisories, and Guidelines Applicable to DNT

Agency	Description	Information	Reference
NATIONAL <i>(cont.)</i>			
b. Water			
EPA	Designated as hazardous substances in accordance with Section 311(b)(2)(A) of the Clean Water Act		EPA 2011h 40 CFR 116.4
	DNT, 2,4-, 2,6-, and 3,4-DNT	Yes	
	Drinking water contaminant candidate list		EPA 1998 63 FR 10274
	2,4-DNT	Yes	
	2,6-DNT	Yes	
	Drinking water standards and health advisories		EPA 2011d
	1-day health advisory for a 10-kg child		
	2,4-DNT	1 mg/L	
	2,6-DNT	0.4 mg/L	
	10-day health advisory for a 10-kg child		
	2,4-DNT	1 mg/L	
	2,6-DNT	0.04 mg/L	
	DWEL		
	2,4-DNT	0.1 mg/L	
	2,6-DNT	0.04 mg/L	
	10 ⁻⁴ Cancer risk		
	2,4-DNT	0.005 mg/L	
	2,6-DNT	0.005 mg/L	
	Master Testing List		EPA 2012c
	2,4-DNT	Yes ^h	
	National primary drinking water standards	No data	EPA 2009a
	National recommended water quality criteria: human health for the consumption of		EPA 2009b
	2,4-DNT		
	Water plus organism	0.11 µg/L ⁱ	
	Organism only	3.4 µg/L	
	Reportable quantities of hazardous substances designated pursuant to Section 311 of the Clean Water Act		EPA 2011k 40 CFR 117.3
	DNT	10 pounds	

8. REGULATIONS, ADVISORIES, AND GUIDELINES

Table 8-1. Regulations, Advisories, and Guidelines Applicable to DNT

Agency	Description	Information	Reference
NATIONAL (cont.)			
c. Food			
FDA	EAFUS ^l	No data	FDA 2012
d. Other			
ACGIH	Carcinogenicity classification DNT	A3 ^{c,l}	ACGIH 2011
EPA	Carcinogenicity classification RfC RfD 2,4-DNT Oral slope factor Identification and listing of hazardous waster 2,4-DNT 2,6-DNT Inert pesticide ingredients in pesticide products Standards for owners and operators of hazardous waste TSD facilities; groundwater monitoring list 2,4-DNT 2,6-DNT Superfund, emergency planning, and community right-to-know Designated CERCLA hazardous substance and reportable quantity DNT and 3,4-DNT 2,4-DNT 2,6-DNT Effective date of toxic chemical release reporting DNT 2,4-DNT 2,6-DNT Extremely hazardous substances and its threshold planning quantity	B2 ^l No data 0.002 mg/kg/day 0.068 (mg/kg/day) ⁻¹¹ U105 U106 No data Yes Yes 10 pounds ^m 10 pounds ⁿ 100 pounds ^o 01/01/1990 01/01/1987 01/01/1987 No data	IRIS 2012 EPA 2011g 40 CFR 261, Appendix VIII EPA 2011j EPA 2011f 40 CFR 264, Appendix IX EPA 2011i 40 CFR 302.4 EPA 2011m 40 CFR 372.65 EPA 2011 40 CFR 355, Appendix A
EPA	TSCA chemical lists and reporting periods 2,4-DNT Effective date Reporting date TSCA health and safety data reporting	 03/11/1994 05/10/1994	EPA 2011 40 CFR 712.30 EPA 2011i 40 CFR 716.120

8. REGULATIONS, ADVISORIES, AND GUIDELINES

Table 8-1. Regulations, Advisories, and Guidelines Applicable to DNT

Agency	Description	Information	Reference
NATIONAL (<i>cont.</i>)			
	2,4-DNT		
	Effective date	03/11/1994	
	Reporting date	06/30/1998	
NTP	Carcinogenicity classification	No data	NTP 2011

^aGroup 2B: possibly carcinogenic to humans

^bGroup 3: not classifiable as to its carcinogenicity to humans

^cTg-DNT is composed primarily of the 2,4- and 2,6-DNT isomers and the other four isomers (2,3-, 2,5-, 3,4-, and 3,5-DNT) make up only 5% of tg-DNT.

^dSkin: refers to the potential significant contribution to the overall exposure by the cutaneous route.

^ePAC-1: mild, transient health effects; PAC-2: irreversible or other serious health effects that could impair the ability to take protective action; PAC-3: life-threatening health effects.

^fNIOSH potential occupational carcinogen

^gSkin designation

^h2,4-DNT was recommended to the MTL by the OECD on the basis of the SIDS. 2,4-DNT was added to the MTL in 1992 and the chemical testing program is currently underway by way of a Voluntary Testing Agreement. The testing needs include health effects, ecological effects, and chemical fate.

ⁱThis criterion is based on carcinogenicity of 10⁻⁶ risk.

^jThe EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS.

^kA3: confirmed animal carcinogen with unknown relevance to humans

^lGroup B2: probable human carcinogen. The carcinogenicity assessment for DNT mixture includes both 2,4- and 2,6-DNT.

^mDesignated CERCLA hazardous substance pursuant to Section 311(b)(2) and Section 307(a) of the Clean Water Act.

ⁿDesignated CERCLA hazardous substance pursuant to Section 311(b)(2) and Section 307(a) of the Clean Water Act, Section 112 of the Clean Air Act, and Section 3001 of RCRA.

^oDesignated CERCLA hazardous substance pursuant to Section 311(b)(2) and Section 307(a) of the Clean Water Act, and Section 3001 of RCRA.

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = acute exposure guideline levels; AIHA = American Industrial Hygiene Association; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DNT = dinitrotoluene; DOE = Department of Energy; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; ERPG = emergency response planning guidelines; FDA = Food and Drug Administration; FR = Federal Register; GRAS = Generally Recognized As Safe; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MTL = Master Testing List; NAAQS = National Ambient Air Quality Standards; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OECD = Organisation for Economic Co-operation and Development; OSHA = Occupational Safety and Health Administration; PAC = Protective Action Criteria; PEL = permissible exposure limit; RCRA = Resource Conservation and Recovery Act; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; SIDS = Screening Information Data Sets; TLV = threshold limit values; TSCA = Toxic Substances Control Act; TSD = treatment, storage, and disposal; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

8. REGULATIONS, ADVISORIES, AND GUIDELINES

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10. GLOSSARY

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

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

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

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

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

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

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

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

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

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

Carcinogen—A chemical capable of inducing cancer.

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

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

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

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Ceiling Value—A concentration of a substance that should not be exceeded, even instantaneously.

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

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

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

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

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

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

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

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

Epidemiology—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

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

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

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

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

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Immunological Effects—Functional changes in the immune response.

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

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

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 that has been reported to have caused death in humans or animals.

Lethal Concentration₍₅₀₎ (LC₅₀)—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 has been reported to have caused death in humans or animals.

Lethal Dose₍₅₀₎ (LD₅₀)—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time₍₅₀₎ (LT₅₀)—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 exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

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

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

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

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

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

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

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

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

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

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

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

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

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

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

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

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

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

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a

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variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

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

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

q_1^* —The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1^* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually $\mu\text{g/L}$ for water, mg/kg/day for food, and $\mu\text{g/m}^3$ for air).

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

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

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

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

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

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

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

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

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

Short-Term Exposure Limit (STEL)—The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value-Time Weighted Average (TLV-TWA) may not be exceeded.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

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

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

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

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

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

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

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

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

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that

APPENDIX A

are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Human Health Sciences, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-57, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,4-DNT
CAS Numbers: 121-14-2
Date: October 2012
Profile Status: Final Draft for Pre-Public Comment
Route: ☐ Inhalation ☒ Oral
Duration: ☒ Acute ☐ Intermediate ☐ Chronic
Graph Key: 10
Species: Dog

Minimal Risk Level: 0.05 ☒ mg/kg/day ☐ ppm

References: Ellis HV, Hong CB, Lee CC, et al. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part I. Beagle dog. J Am Coll Toxicol 4:233-242.

U.S. Army. 1978a. Mammalian toxicity of munitions compounds. Phase I. Acute oral toxicity, primary skin and eye irritation, dermal sensitization, disposition and metabolism and Ames tests of additional compounds. Progress report no. 6. Fort Detrick, MD: U.S. Army, Medical Bioengineering Research and Development Laboratory. ADA069333.

Experimental design: In a subchronic study, beagle dogs (4/sex/group) were administered 0, 1, 5, or 25 mg/kg/day 2,4-DNT for up to 13 weeks. 2,4-DNT was mixed with lactose and administered as capsules. Dogs were observed daily for behavioral changes and clinical signs of toxicity. Body weights were recorded weekly. Blood was taken before initiation of treatment and at 4, 8, and 13 weeks for evaluation of hematological parameters (erythrocyte, reticulocyte, platelet, and total and differential leukocyte counts; Heinz bodies; hematocrit, hemoglobin, and methemoglobin concentrations; mean cell volume, hemoglobin, and hemoglobin concentration) and clinical chemistry analyses (glucose, urea nitrogen, sodium, potassium, calcium, magnesium, and chloride; and the serum enzyme activity of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase). Animals (1 sex/group) were sacrificed after 4 or 13 weeks of continuous treatment; an additional dog/sex/group was discontinued treatment after 4 or 13 weeks and sacrificed after 4 weeks of recovery. The two high-dose dogs removed from treatment at 4 weeks were not sacrificed until 8 months after cessation of treatment to test the reversibility of effects after a longer recovery period. When animals were sacrificed moribund or at study termination, they were examined for gross lesions. Major organs and tissues (heart, liver, spleen, kidneys, adrenals, and gonads) were weighed; "various" tissues (not specified) were subjected to histopathology. To evaluate the immunologic response to 2,4-DNT, the concentration of IgE in the serum was assessed after treatment for 4, 8, or 13 weeks or after treatment for 4 or 13 weeks followed by recovery for 4 weeks. Bone marrow and kidney cultures were also maintained and cytogenetic analyses (evaluation of chromosome number and morphology) were performed.

Effects noted in study and corresponding doses: Data for acute-duration oral exposure were obtained from daily cageside observations for behavioral changes and clinical signs of toxicity during the first 14 days of treatment. No mortality was observed during the first 14 days of treatment. No behavioral changes or clinical signs of toxicity were observed in dogs treated with 1 or 5 mg/kg/day. Neurotoxicity was observed at 25 mg/kg/day. Evidence of neurotoxicity, identified as loss of hind leg control, was first observed in a female dog on day 12 of treatment. Three additional male dogs showed similar signs on day 14 of treatment. All high-dose dogs showed signs of neurotoxicity after treatment for 12–22 days. The onset and severity of toxic signs reportedly varied among dogs within the same treatment group; some dogs were moribund at the same time that others began experiencing symptoms. In individual dogs, symptom severity varied over time, with no duration-related pattern of severity. Although incidence data

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were not reported, the study authors noted that the neurotoxic effects most often observed in the 13-week study were incoordination of the hind legs and stiffness that produced an abnormal hopping gait. Some dogs experienced paralysis of the hind legs. In severe cases, stiffness progressed from the hind legs to the trunk, forelegs, neck, and head. Histopathological assessments conducted at 4 or 13 weeks showed lesions of the central nervous system, including generalized vacuolization, hypertrophy, mitosis of the endothelium and focal gliosis in the cerebellum, and/or perivascular hemorrhage in the cerebellum and brain stem in high-dose animals (2/2 and 3/3 animals evaluated at 4 and 13 weeks, respectively). However, the study authors noted that the most severe of these lesions occurred in dogs that developed toxic signs of neurotoxicity late in the study (time to onset of symptoms not reported). Results of this study identify acute-duration NOAEL and LOAEL values for neurotoxicity in dogs of 5 and 25 mg/kg/day, respectively.

Dose and end point used for MRL derivation:

[X] NOAEL [] LOAEL 5 mg/kg/day was the NOAEL for neurological effects (loss of hind leg control).

The NOAEL value of 5 mg/kg/day for neurotoxicity in dogs was identified as the POD for derivation of the acute-duration oral MRL for 2,4-DNT (Ellis et al. 1985; U.S. Army 1978b). Neurotoxicity data were not suitable for BMD modeling, since effects were only observed at the highest dose tested. Therefore, the NOAEL value for 5 mg/kg/day was used at the POD. This value was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability), resulting in an acute-duration oral MRL for 2,4-DNT of 0.05 mg/kg/day.

Uncertainty Factors used in MRL derivation:

- [] 10 for use of a LOAEL
- [X] 10 for extrapolation from animals to humans
- [X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: No other acute-duration studies were located in which neurotoxicity was reported after oral exposure to 2,4-DNT. Other acute-duration studies in rodents identified hepatic effects (decreased serum albumin and gene expression changes) and hematological effects (erythrocytosis) in female Sprague-Dawley rats treated at 99 mg/kg/day via gavage and observed for 24 or 48 hours (Deng et al. 2011), slight cyanosis in male Sprague-Dawley rats treated at 60 mg/kg (the lowest tested dose) for 5 days (Lane et al. 1985), and decreased fertility in CD-1 female mice dosed with 250 mg/kg 2,4-DNT for 2 days (Soares and Lock 1980). Neurotoxicity was observed in beagle dogs after subchronic or chronic treatment with 2,4-DNT. Clinical signs of neurotoxicity (including incoordination and paralysis), sometimes accompanied by central nervous system lesions (generalized vacuolization, hypertrophy, mitosis of the endothelium and focal gliosis in the cerebellum, and perivascular hemorrhages of the cerebellum and brain stem) were reported in dogs (4/sex/group) dosed with 25 mg/kg 2,4-DNT for up to 13 weeks (Ellis et al. 1985; U.S. Army 1978b) and in dogs (6 sex/group) treated at 1.5 (one dog) or 10 mg/kg/day (all dogs) for up to 24 months (Ellis et al. 1985; U.S. Army 1979). Dogs appear to be the most sensitive species for 2,4-DNT-induced neurotoxicity; in CD rats and CD-1 mice treated with 2,4-DNT for up to 24 months,

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neurotoxic effects were absent or occurred at much higher doses in similarly designed studies (U.S. Army 1978b, 1979). Neuromuscular effects similar to those observed in dogs occurred in rats administered 2,4-DNT at 266 or 145 mg/kg/day (for males and females, respectively) for up to 13 weeks, but not in rats treated with 2,4-DNT at up to 34 or 45 mg/kg/day (for males and females, respectively) for 24 months. Mice treated with 2,4-DNT at 413 or 468 mg/kg/day (for males and females, respectively) for up to 13 weeks or 898 mg/kg/day for 24 months did not show clinical signs of neurotoxicity (U.S. Army 1978b, 1979).

Agency Contact (Chemical Manager): Carolyn Harper

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MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,4-DNT
CAS Numbers: 121-14-2
Date: October 2012
Profile Status: Final Draft for Pre-Public Comment
Route: ☐ Inhalation ☒ Oral
Duration: ☐ Acute ☒ Intermediate ☐ Chronic
Graph Key: 27
Species: Dog

Minimal Risk Level: 0.007 ☒ mg/kg/day ☐ ppm

References: U.S. Army. 1979. Mammalian toxicity of munitions compounds. Phase III: Effects of lifetime exposure. Part I. 2,4-Dinitrotoluene. Final report no. 7. Fort Detrick, MD: U.S. Army and Medical Bioengineering Research Development Laboratory. ADA077692.

Ellis HV, Hong CB, Lee CC, et al. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part I. Beagle dog. J Am Coll Toxicol 4:233-242.

Experimental design: Young beagle dogs (6 dogs/sex/group; age not specified) were administered 0, 0.2, 1.5, or 10 mg/kg 2,4-DNT in capsules for 24 months. Dogs were observed daily for behavioral changes and clinical signs of toxicity. Body weights were recorded weekly. Feed consumption was measured during 1 week each month starting in month 6. Blood was taken before initiation of treatment and after 3, 6, 9, 12, 18, and 24 months of exposure for assessment of hematological parameters (erythrocyte, reticulocyte, platelet, and total and differential leukocyte counts; Heinz bodies, clotting time, hematocrit, hemoglobin, and methemoglobin concentrations; and mean cell volume, hemoglobin, and hemoglobin concentration) and clinical chemistry (fasting glucose, urea nitrogen, levels of sodium, potassium, calcium, magnesium, chloride, and bilirubin [high-dose dogs with toxic signs], and the serum enzyme activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) analyses. Animals (one male and one female/group) were sacrificed after 12 or 24 months of continuous treatment; an additional dog/sex/group was discontinued from treatment at these time points and were sacrificed after a 4-week recovery period to evaluate the reversibility of effects (including clinical signs, hematology and clinical chemistry, organ weights, and histopathological effects). Animals that were moribund during the study and those that survived to study termination were sacrificed and examined for gross lesions; major organs and tissues (including the brain, heart, liver, spleen, kidneys, adrenals, thyroids, pituitary, and gonads) were weighed, and comprehensive histopathological analyses (35 tissues) were performed.

Effects noted in study and corresponding doses: Intermediate-duration oral exposure of dogs to 2,4-DNT produced methemoglobinemia, anemia, and compensatory hematopoiesis (Ellis et al. 1985; U.S. Army 1979). Hematological effects of 2,4-DNT are initiated by methemoglobin production, which occurs when the ferrous iron in complex with the heme groups of hemoglobin is oxidized to ferric iron. Ferric iron does not bind oxygen, resulting in anemia. Ferric iron also contributes to the denaturation of hemoglobin and subsequent removal of erythrocytes from the blood. Heinz bodies are also detected as granules in erythrocytes resulting from denatured hemoglobin. Increased hematopoiesis is typically observed as a compensatory response to decreased erythrocyte count. Hematological effects consistent with development of methemoglobinemia were observed in dogs administered oral 2,4-DNT at all intermediate duration time points (3, 6, and 9 months). Although effects at all time points were qualitatively similar, hematological changes observed after 9 months of exposure were more consistent and pronounced than those observed at the 3- and 6-month time periods. Therefore, only data from the 9-month evaluation were considered for derivation of the intermediate-duration oral MRL. The only significant effects

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observed at the intermediate-duration time points were changes to hematological parameters. At the mid- and low dose, no clinical signs of toxicity, behavioral changes, or effects on clinical chemistry parameters were observed at the intermediate-duration time points at any of the doses tested. At the high dose, clinical signs of neurotoxicity (decreased muscle control and incoordination), sometimes accompanied by decreased body weight, were observed; these effects contributed to the death of four of six dogs within the first 20 weeks of the study.

Effects on hematological parameters in male and female dogs administered oral 2,4-DNT for 9 months are summarized in Table A-1. Male and female dogs exposed to 2,4-DNT for 9 months at doses of 1.5 and 10 mg/kg/day showed detectable amounts of methemoglobin in the serum (the initiating hematological effect), with changes reaching statistical significance in males and females in the 10 mg/kg/day group. In female dogs administered 10 mg/kg/day, statistically significant decreases in erythrocyte count, hematocrit, and hemoglobin, a statistically significant increase in reticulocyte count, and the presence of Heinz bodies in serum were observed. Similar hematological effects were observed in female dogs administered 0.2 and 1.5 mg/kg/day, although effects did not reach statistical significance, most likely because the power of the study to detect statistically significant changes was compromised by the small number of dogs per treatment group. However, based on a clinically significant increase in methemoglobin levels of 225% in female dogs administered 1.5 mg/kg/day, the NOAEL and LOAEL values for hematological effects in this study are 0.2 and 1.5 mg/kg/day, respectively. Effects on hematological parameters in male dogs were similar to those in female dogs, although changes did not reach statistical significance in the 10 mg/kg/day group, possibly due to low numbers of male dogs evaluated (hematological data available for only two males in the 10 mg/kg/day group). After treatment for 18 or 24 months, slight or no anemia, near-normal reticulocyte levels, no Heinz bodies, and minimal amounts of methemoglobin were detected, likely reflective of an adaptive response. Recovery from hematological effects also occurred in dogs allowed to recover for 4 weeks after dosing for 12 or 24 months.

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Table A-1. Hematological Effects in Beagle Dogs Exposed to 2,4-DNT for 9 Months

End point	Dose (mg/kg/day)			
	0	0.2	1.5	10
Males				
Erythrocyte count (x10/mm)	6.51±0.12 (6) ^a	6.23±0.18 (6) [↓4]	6.36±0.13 (6) [↓2]	6.36±0.13 (2) [↓2]
Heinz bodies (%)	0.0±0.0(6)	0.0±0.0(6) [NA]	0.0±0.0(6) [NA]	1.8±0.6(2) ^b [NA]
Reticulocytes (%)	0.69±0.15 (6)	0.55±0.09 (6) [↓20]	0.57±0.11 (6) [↓17]	1.39±0.47 (2) [↑101]
Hematocrit (%)	46.7±1.0 (6)	45.3±1.1 (6) [↓3]	45.0±0.9 (6) [↓4]	42.0±3.0 (2) [↓10]
Hemoglobin (%)	16.6±0.3 (6)	15.4±0.4 (6)	15.7±0.3 (6)	15.7±0.3 (6)
Methemoglobin (%)	0.0±0.0 (6)	0.0±0.0 (6) [NA]	0.9±0.6 (6) [NA]	2.8±0.3 (2) ^b [NA]
Females				
Erythrocyte count (x10/mm)	6.49±0.24 (6) ^a	5.90±0.17 (6) [↓9]	5.78±0.21 (6) [↓11]	5.05±0.17 (6) ^b [↓22]
Heinz bodies (%)	0.0±0.0(6)	0.0±0.0(6) [NA]	0.0±0.0(6) [NA]	0.84±0.21 (6) ^b [NA]
Reticulocytes (%)	0.60±0.08 (6)	0.70±0.17 (6) [↑17]	0.45±0.10 (6) [↓25]	1.33±0.19 (6) ^b [↑122]
Hematocrit (%)	46.0±1.3 (6)	42.8±1.7 (6) [↓7]	42.5±1.2 (6) [↓8]	37.2±1.6 (6) ^b [↓19]
Hemoglobin (%)	16.1±0.5 (6)	14.7±0.6 (6) [↓9]	14.8±0.5 (6) [↓8]	13.3±0.5 (6) ^b [↓17]
Methemoglobin (%)	0.4±0.4 (6)	0.0±0.0 (6) [↓100]	1.3±0.6 (6) [↑225]	2.8±0.7 (6) ^b [↑600]

^aValues are means±standard error (number of animals) [percent change from controls].

^bStatistically significant based on analyses performed by the study authors (Dunnett's multiple comparison procedure).

DNT = dinitrotoluene; NA = not applicable

Sources: Ellis et al. 1985; U.S. Army 1979

Dose and end point used for MRL derivation:

[] NOAEL [] LOAEL [X] BMDL 0.67 mg/kg/day as a BMDL_{1SD} for hematological effects (decreased hematocrit)

Results of hematology assessments show that intermediate-duration, oral exposure of dogs to 2,4-DNT induced methemoglobinemia, anemia, and compensatory hematopoiesis (Ellis et al. 1985; U.S. Army 1979). Changes in several hematological parameters, including decreased erythrocyte count, hematocrit, and hemoglobin and increased reticulocytes, methemoglobin, and Heinz bodies were observed after treatment with 2,4-DNT for 3, 6, and 9 months, (Table A-1). However, hematological effects at 9 months were more pronounced and consistent than those observed at 3 and 6 months; therefore, hematological

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effects observed at 9 months were identified as the critical effect for derivation of the intermediate-duration oral MRL. To determine the POD, hematological data from female dogs treated with 2,4-DNT for 9 months were further evaluated by BMD analysis. The following data sets in female dogs were selected for BMD modeling: erythrocyte count, reticulocytes, hematocrit, hemoglobin, and methemoglobin. Data on Heinz bodies in serum were not selected for BMD modeling, since these granules were detected in high-dose animals only (i.e., all-or-nothing response); the absence of changes at lower dose levels suggests that these data would not be suitable for modeling. Hematological data from male dogs were not considered for additional BMD analyses due to the low number of dogs evaluated in the 10 mg/kg/day group (for most hematological parameters, data were available for only two dogs).

To determine the POD for derivation of the intermediate-duration oral MRL, all available continuous-variable models in the EPA BMDS (version 2.1) were fit to the data for increased methemoglobin, increased reticulocytes, decreased hemoglobin, decreased erythrocyte count, and decreased hematocrit (Ellis et al. 1985; U.S. Army 1979). The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls, and are in units of mg/kg-day. For continuous data, in the absence of a clear criteria as to what level of change should be considered adverse, the BMR is defined as a change equal to 1 standard deviation from the control mean (EPA 2000a). Adequate model fit is judged by three criteria: goodness-of-fit ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest benchmark dose (BMDL, the lower limit of a one-sided 95% confidence interval on the BMD) is selected as the POD when differences between the BMDLs estimated from these models are >3 -fold; otherwise, the BMDL from the model with the lowest AIC is chosen.

Neither the constant nor the non-constant variance model provided an adequate fit to the data for decreased erythrocyte count or increased methemoglobin; therefore, these data were not considered suitable for BMD modeling. BMD model prediction for increased reticulocytes, decreased hemoglobin, and decreased hematocrit are shown in Tables A-2, A-3, and A-4, respectively. Of models meeting adequate fit criteria for each hematological parameter, the lowest BMDL_{1SD} values were 5.64 mg/kg/day for increased reticulocytes (polynomial 3-degree; Figure A-1), 3.66 mg/kg/day for decreased hemoglobin (exponential model 2; Figure A-2), and 0.67 mg/kg/day for decreased hematocrit (exponential model 4; Figure A-3). Of these, the lowest BMDL_{1SD} of 0.67 mg 2,4-DNT/kg/day for decreased hematocrit was selected as the POD for derivation of the intermediate-duration oral MRL for 2,4-DNT. This value was divided by an uncertainty factor of 100 (10 for animals to human extrapolation and 10 for human variability) resulting in an intermediate-duration oral MRL of 0.007 mg/kg/day.

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Table A-2. Model Predictions for 2,4-DNT for Increased Reticulocytes (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)

Model	Test for significant difference p-value ^a	Variance p-value ^b	Means p-value ^b	Scaled residuals ^c				BMD _{1SD} (mg/kg-day)	BMDL _{1SD} (mg/kg-day)
				Dose below BMD	Dose above BMD	Overall largest	AIC		
Constant variance									
Exponential (model 2) ^d	0.0007	0.12	0.23	-1.32	0.09	-1.32	-21.60	5.55	4.35
Exponential (model 3) ^d	0.0007	0.12	0.18	-0.98	5.59x10 ⁻⁸	-0.98	-20.75	9.40	4.58
Exponential (model 4) ^d	0.0007	0.12	0.06	-1.51	0.21	-1.50	-18.93	4.61	3.19
Exponential (model 5) ^d	0.0007	0.12	NA	-0.98	8.61x10 ⁻⁸	-0.98	-18.75	9.17	1.59
Hill ^d	0.0007	0.12	NA	-0.98	-9.04x10 ⁻⁷	-0.98	-18.75	9.10	1.60
Linear ^e	0.0007	0.12	0.17	-1.51	0.21	-1.51	-20.93	4.61	3.19
Polynomial (2-degree) ^e	0.0007	0.12	0.36	-1.06	0.02	-1.06	-22.50	6.69	5.60
Polynomial (3-degree)^{e,f}	0.0007	0.12	0.40	-0.99	0.003	-0.99	-22.72	7.64	5.64
Power ^d	0.0007	0.12	0.18	-0.98	3.56x10 ⁻¹⁰	-0.98	-20.75	9.31	3.68

^aValues >0.05 fail to meet conventional goodness-of-fit criteria.

^bValues <0.10 fail to meet conventional goodness-of-fit criteria.

^cScaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.

^dPower restricted to ≥1.

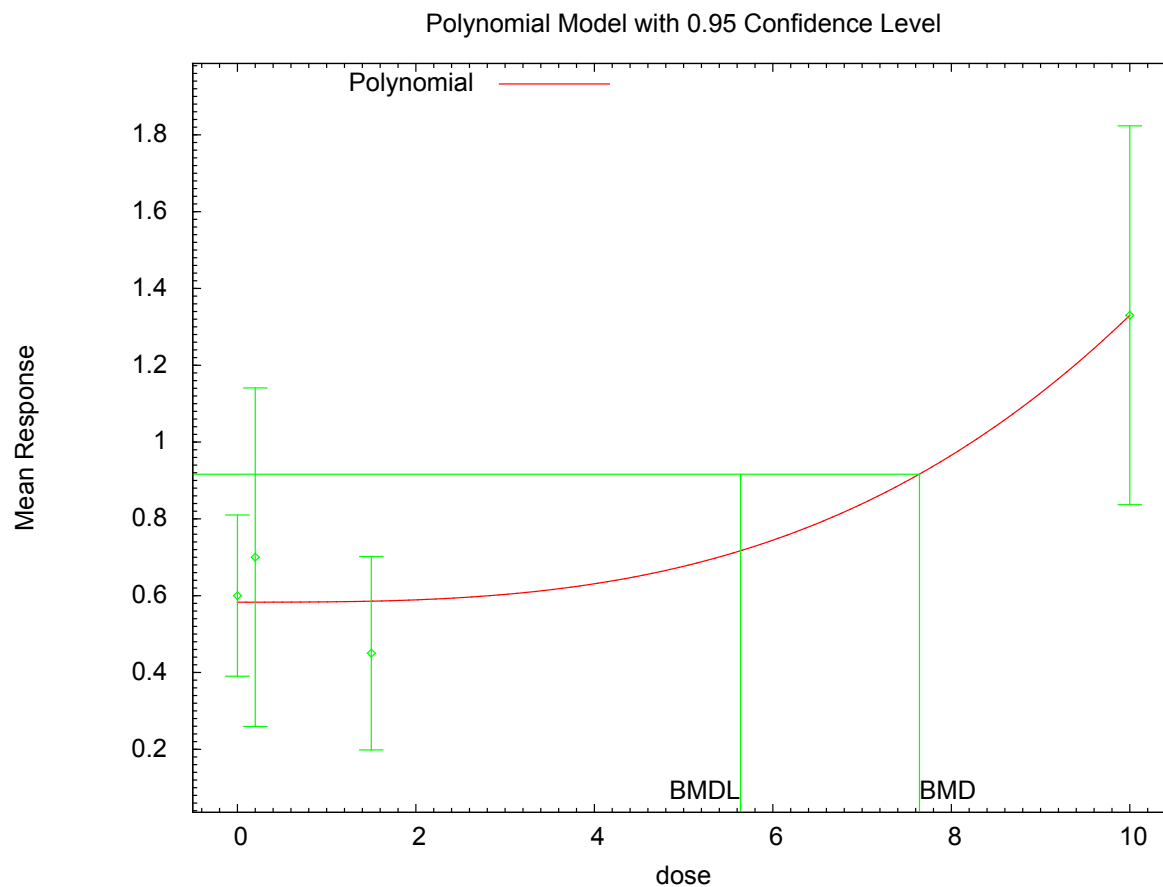
^eCoefficients restricted to be positive.

^fSelected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, all models, except for the Exponential 4 and 5 and Hill models, provided adequate fit to means. BMDLs for models providing adequate fit were considered to be sufficiently close (differed by <2–3-fold), so the model with the lowest AIC was selected.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., ₁₀ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

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Figure A-1. Fit of Polynomial 3-Degree Model to Data on 2,4-DNT for Increased Reticulocytes (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)



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Table A-3. Model Predictions for 2,4-DNT for Decreased Hemoglobin (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)

Model	Test for significant difference p-value ^a	Variance p-value ^b	Means p-value ^b	Scaled residuals ^c			AIC	BMD _{1SD} (mg/kg-day)	BMDL _{1SD} (mg/kg-day)
				Dose below BMD	Dose above BMD	Overall largest			
Constant variance									
Exponential (model 2)^{d,e}	0.04	0.92	0.14	-0.40	0.10	1.46	41.47	5.90	3.66
Exponential (model 3) ^d	0.04	0.92	0.14	-0.40	0.10	1.46	41.47	5.90	3.66
Exponential (model 4) ^d	0.04	0.92	0.07	0.29	-0.03	-1.36	42.88	2.43	0.01
Exponential (model 5) ^d	0.04	0.92	0.07	0.29	-0.03	-1.36	42.88	2.43	0.01
Hill ^d	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linear ^f	0.04	0.92	0.14	-0.43	0.09	1.48	41.52	6.12	3.94
Polynomial (2-degree) ^f	0.04	0.92	0.14	-0.43	0.09	1.48	41.52	6.12	3.94
Polynomial (3-degree) ^f	0.04	0.92	0.14	-0.43	0.09	1.48	41.52	6.12	3.94
Power ^d	0.04	0.92	0.14	-0.43	0.09	1.48	41.52	6.12	3.94

^aValues >0.05 fail to meet conventional goodness-of-fit criteria.

^bValues <0.10 fail to meet conventional goodness-of-fit criteria.

^cScaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.

^dPower restricted to ≥1.

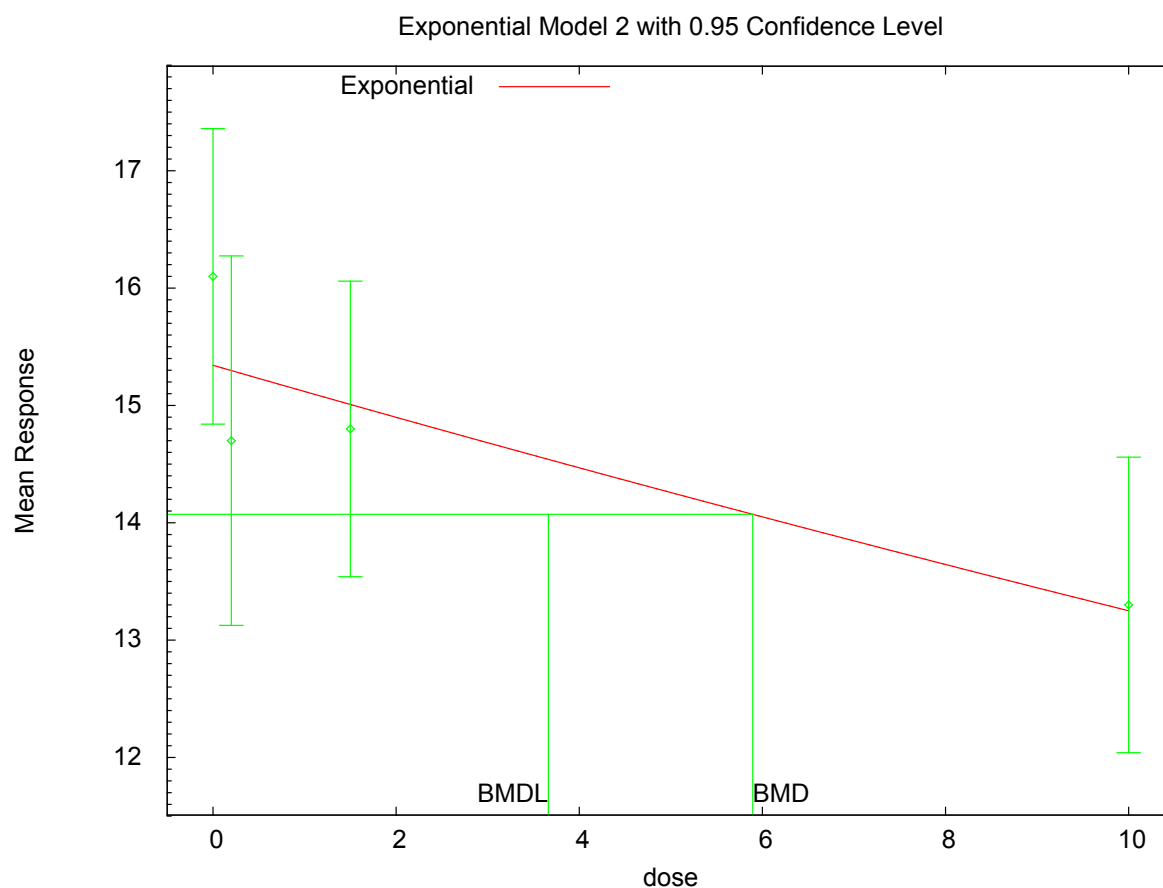
^eSelected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, all models, except for the Exponential 4 and 5 and Hill model (computation failed), provided adequate fit to means. BMDLs for models providing adequate fit were considered to be sufficiently close (differed by <2–3-fold), so the model with the lowest AIC was selected (the Exponential 3 model converged on to the Exponential 2).

^fCoefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., ₁₀ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

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Figure A-2. Fit of the Exponential 2 Model to Data on 2,4-DNT for Decreased Hemoglobin (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)



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Table A-4. Model Predictions for 2,4-DNT for Decreased Hematocrit (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)

Model	Test for significant difference p-value ^a	Variance p-value ^b	Means p-value ^b	Scaled residuals ^c				BMD _{1SD} (mg/kg-day)	BMDL _{1SD} (mg/kg-day)
				Dose below BMD	Dose above BMD	Overall largest	AIC		
Constant variance									
Exponential (model 2) ^d	0.01	0.79	0.26	-0.42	0.09	1.24	89.65	4.60	3.04
Exponential (model 3) ^d	0.01	0.79	0.26	-0.42	0.09	1.24	89.65	4.60	3.04
Exponential (model 4)^{d,e}	0.01	0.79	0.14	0.21	-0.01	-1.10	91.12	2.19	0.67
Exponential (model 5) ^d	0.01	0.79	0.14	0.21	-0.01	-1.10	91.12	2.19	0.67
Hill ^d	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linear ^f	0.01	0.79	0.25	-0.45	0.09	1.26	89.71	4.86	3.32
Polynomial (2-degree) ^f	0.01	0.79	0.25	-0.45	0.09	1.26	89.71	4.86	3.32
Polynomial (3-degree) ^f	0.01	0.79	0.25	-0.45	0.09	1.26	89.71	4.86	3.32
Power ^d	0.01	0.79	0.25	-0.45	0.09	1.26	89.71	4.86	3.32

^aValues >0.05 fail to meet conventional goodness-of-fit criteria.

^bValues <0.10 fail to meet conventional goodness-of-fit criteria.

^cScaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.

^dPower restricted to ≥1.

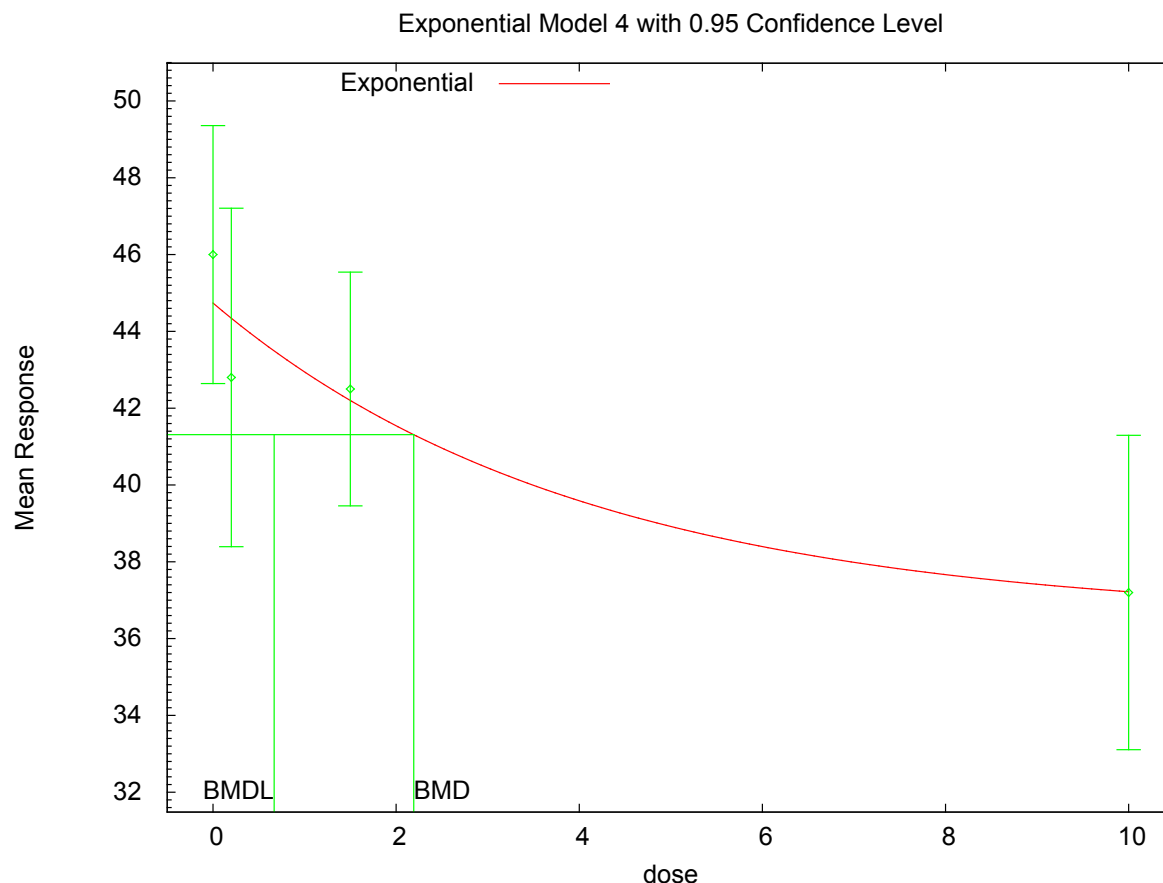
^eSelected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, all models, except for the Hill model (computation failed), provided adequate fit to means. BMDLs for models providing adequate fit were not considered to be sufficiently close (differed by >2–3-fold), so the model with the lowest BMDL was selected (the Exponential 5 model converged on to the Exponential 4).

^fCoefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., ₁₀ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

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Figure A-3. Fit of the Exponential 4 Model to Data on 2,4-DNT for Decreased Hematocrit (%) in Female Dogs Following 9 Months of Exposure (U.S. Army 1979)



08:07 04/12 2012

Uncertainty Factors used in MRL derivation:

- [] 10 for use of a LOAEL
- [X] 10 for extrapolation from animals to humans
- [X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: The hematological effects observed in this study are consistent with well-characterized effects observed after exposure to aromatic amines and with effects observed at higher doses in other studies of intermediate duration (Hong et al. 1985; Lee et al. 1985; Kozuka et al. 1979; U.S. Army 1978b). Similar hematological effects (anemia, accompanied by the presence of Heinz bodies) were observed in beagle dogs treated at 25 mg/kg/day (but not 5 mg/kg/day) for up to 13 weeks (Ellis et al. 1985; U.S. Army 1978b). Dogs

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appear to be the most sensitive species. In Wistar rats, methemoglobin was increased substantially after treatment with 2,4-DNT at 347 mg/kg/day for 6 months (Kozuka et al. 1979). Milder hematological effects (mild reticulocytosis and hemosiderosis of the spleen) were also observed in CD rats treated at 93 or 108 mg/kg/day (for males or females, respectively) for up to 13 weeks (Lee et al. 1985; U.S. Army 1978b). Mice treated with 2,4-DNT for up to 13 weeks showed evidence of hematological effects (mild anemia, characterized by increased reticulocytes and decreased hematocrit and hemoglobin) only at the highest tested dose (413 mg/kg/day for males or 468 mg/kg/day for females) (Ellis et al. 1985; U.S. Army 1978b). Hematological effects were also observed in 2-year studies in beagle dogs, CD rats, and CD-1 mice, with dogs being the most sensitive species. Female dogs treated with 2,4-DNT at 1.5 mg/kg/day showed decreased erythrocyte count, hematocrit, and hemoglobin after 12 months; similar effects were observed in dogs of both sexes at 10 mg/kg/day (U.S. Army 1979). Anemia occurred at higher doses in 2-year studies in rats (≥ 3.9 mg/kg/day) and mice (898 mg/kg/day).

Agency Contact (Chemical Manager): Carolyn Harper

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MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,4-DNT
CAS Numbers: 121-14-2
Date: October 2012
Profile Status: Final Draft for Pre-Public Comment
Route: ☐ Inhalation ☒ Oral
Duration: ☐ Acute ☐ Intermediate ☒ Chronic
Graph Key: 52
Species: Dog

Minimal Risk Level: 0.001 ☒ mg/kg/day ☐ ppm

References: U.S. Army. 1979. Mammalian toxicity of munitions compounds. Phase III: Effects of lifetime exposure. Part I. 2,4-Dinitrotoluene. Final report no. 7. Fort Detrick, MD: U.S. Army and Medical Bioengineering Research Development Laboratory. ADA077692.

Ellis HV, Hong CB, Lee CC, et al. 1985. Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part I. Beagle dog. J Am Coll Toxicol 4:233-242.

Experimental design: Young beagle dogs (6 dogs/sex/group; age not specified) were administered 0, 0.2, 1.5, or 10 mg/kg/day 2,4-DNT in capsules for 24 months. Dogs were observed daily for behavioral changes and clinical signs of toxicity. Body weights were recorded weekly. Feed consumption was measured during 1 week each month starting in month 6. Blood was taken before initiation of treatment and after 3, 6, 9, 12, 18, and 24 months of exposure for assessment of hematological parameters (erythrocyte, reticulocyte, platelet, and total and differential leukocyte counts; Heinz bodies; clotting time, hematocrit, hemoglobin, and methemoglobin concentrations; and mean cell volume, hemoglobin, and hemoglobin concentration) and clinical chemistry (fasting glucose, urea nitrogen, sodium, potassium, calcium, magnesium, chloride, and bilirubin [high-dose dogs with toxic signs], and the serum enzyme activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) analyses. Animals (one male and one female/group) were sacrificed after 12 or 24 months of continuous treatment; an additional dog/sex/group was discontinued from treatment at these time points and were sacrificed after a 4-week recovery period to evaluate the reversibility of effects (including clinical signs, hematology and clinical chemistry, organ weights, and histopathological analyses). Animals that were moribund during the study and those that survived to study termination were sacrificed and examined for gross lesions; major organs and tissues (including the brain, heart, liver, spleen, kidneys, adrenals, thyroids, pituitary, and gonads) were weighed, and comprehensive histopathological analyses (35 tissues) were performed.

Effects noted in study and corresponding doses: Chronic-duration oral exposure of dogs to 2,4-DNT at ≥ 1.5 mg/kg/day produced anemia and compensatory hematopoiesis (Ellis et al. 1985; U.S. Army 1979). Hematological effects of 2,4-DNT are initiated by methemoglobin production, which occurs when the ferrous iron in complex with the heme groups of hemoglobin is oxidized to ferric iron. Ferric iron does not bind oxygen, resulting in anemia. Ferric iron also contributes to the denaturation of hemoglobin and subsequent removal of erythrocytes from the blood. Heinz bodies (granules of denatured hemoglobin) are also detected within erythrocytes. Increased hematopoiesis is typically observed as a compensatory response to decreased erythrocyte count.

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Hematological effects consistent with the development of methemoglobin-induced anemia and compensatory hematopoiesis were observed after dosing for 12 months in dogs administered 1.5 and 10 mg/kg/day (Table A-5). Female dogs administered 2,4-DNT at 1.5 mg/kg/day for 12 months showed statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin concentration after treatment. At 10 mg/kg/day, more pronounced changes in these hematological parameters were observed, with statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and a statistically significant increase in reticulocyte count. In the low-dose group, similar hematological effects (decreased erythrocyte count and decreased hematocrit) were observed in female dogs, but these changes were not statistically significant. Effects on hematological parameters in male dogs were similar to those seen in female dogs, although many changes (with the exception of reticulocytes) did not reach statistical significance in the 10 mg/kg/day group, possibly due to low numbers of male dogs evaluated (hematological data available for only two males in the 10 mg/kg/day group). After treatment for 18 or 24 months in both males and females, only slight or no anemia, near normal reticulocyte levels, no Heinz bodies, and minimal amounts of methemoglobin were detected, likely reflective of an adaptive response. Therefore, only data from the 12-month evaluation were considered for derivation of the chronic-duration oral MRL.

No clinical signs of toxicity, behavioral changes, or effects on hematological or clinical chemistry parameters were observed in dogs administered 2,4-DNT at 0.2 mg/kg/day. Four high-dose dogs (three males and one female) exhibited severe signs of neurotoxicity (characterized by decreased muscle control and incoordination), sometimes accompanied by a reduction in body weight. These effects contributed to the death of three of six high-dose dogs (all males) prior to study termination (study weeks 8–20). Clinical signs of neurotoxicity were also noted intermittently in one male dog administered 2,4-DNT at 1.5 mg/kg/day. Although biliary hyperplasia was noted at necropsy in male and female dogs administered 2,4-DNT, the frequency of the response did not exhibit dose-dependence.

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Table A-5. Hematological Effects in Beagle Dogs Exposed to 2,4-DNT for 12 Months

End point	Dose (mg/kg/day)			
	0	0.2	1.5	10
Males				
Erythrocyte count (x10/mm)	5.96±0.22 (6) ^a	5.33±0.16 (6) [↓11]	5.69±0.19 (6) [↓5]	5.22±0.19 (2) [↓12]
Heinz bodies (%)	0.0±0.0(6)	0.0±0.0(6) [NA]	0.0±0.0(6) [NA]	0.52±0.37 (2) ^b [NA]
Reticulocytes (%)	0.40±0.09 (6)	0.78±0.13 (6) [↑95]	0.66±0.11 (6) [↑65]	1.23±0.23 (2) ^b [↑208]
Hematocrit (%)	45.2±0.9 (6)	41.8±1.2 (6) [↓8]	44.7±0.8 (6) [↓1]	44.0±4.0 (2) [↓3]
Hemoglobin (%)	15.1±0.4 (6)	14.1±0.3 (6) [↓7]	14.8±0.4 (6) [↓2]	14.2±1.2 (2) [↓6]
Methemoglobin (%)	0.0±0.0 (6)	0.0±0.0 (6) [NA]	0.0±0.0 (6) [NA]	0.0±0.0 (2) [NA]
Females				
Erythrocyte count (x10/mm)	5.87±0.20 (6) ^a	5.54±0.14 (6) [↓6]	4.69±0.25 (6) ^b [↓20]	4.45±0.26 (6) ^b [↓24]
Heinz bodies (%)	0.0±0.0(6)	0.0±0.0(6) [NA]	0.0±0.0(6) [NA]	0.5±0.2 (6) ^b [NA]
Reticulocytes (%)	0.39±0.04 (6)	0.84±0.07 (6) ^b [↑115]	0.45±0.06 (6) [↑15]	1.59±0.18 (6) ^b [↑308]
Hematocrit (%)	45.8±1.1 (6)	44.7±1.3 (6) [↓2]	41.8±1.2 (6) ^b [↓9]	40.8±0.6 (6) ^b [↓11]
Hemoglobin (%)	15.1±0.4 (6)	15.1±0.4 (6) [0]	13.7±0.8 (6) ^b [↓9]	12.9±0.2 (6) ^b [↓15]
Methemoglobin (%)	0.0±0.0 (6)	0.6±0.6 (6) [NA]	0.0±0.0 (6) [NA]	0.0±0.0 (6) [NA]

^aValues are means±standard error (number of animals) [percent change from controls].

^bStatistically significant based on analyses performed by the study authors (Dunnett's multiple comparison procedure).

DNT = dinitrotoluene; NA = not applicable

Sources: Ellis et al. 1985; U.S. Army 1979

Dose and end point used for MRL derivation:

[] NOAEL [] LOAEL [X] BMDL 0.12 mg/kg was the BMDL_{1SD} for hematological effects (decreased erythrocyte count).

Results of hematology assessments show that chronic-duration, oral exposure of dogs to 2,4-DNT induced anemia and compensatory hematopoiesis (Ellis et al. 1985; U.S. Army 1979). Changes in several hematological parameters, including decreased erythrocyte count, hematocrit, and hemoglobin were observed after treatment with 2,4-DNT at 1.5 mg/kg/day for 12 months (Table A-3). Hematological effects were selected as the critical effect rather than neurotoxicity, which was observed only intermittently in one of six dogs exposed to 1.5 mg/kg/day. Hematological data are expressed as group means; therefore, these data are considered more robust than observations of intermittent neurotoxicity in

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a single animal. To determine the POD for derivation of the chronic-duration oral MRL for 2,4-DNT, hematological data from female dogs treated with 2,4-DNT for 12 months were further evaluated by BMD analysis. The following data sets in female dogs were selected for BMD modeling: erythrocyte count, hematocrit, and hemoglobin. Hematological data from male dogs were not considered for additional BMD analyses due to the low number of dogs evaluated in the 10 mg/kg/day group (data were available for only two dogs). All available continuous-variable models in the EPA BMDS (version 2.1) were fit to the data. The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls, and are in units of mg/kg-day. For continuous data, in the absence of a clear criteria as to what level of change should be considered adverse, the BMR is defined as a change equal to 1 standard deviation from the control mean (EPA 2000a). Adequate model fit is judged by three criteria: goodness-of-fit ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest benchmark dose (BMDL, the lower limit of a one-sided 95% confidence interval on the BMD) is selected as the POD when differences between the BMDLs estimated from these models are >3 -fold; otherwise, the BMDL from the model with the lowest AIC is chosen.

Neither the constant nor the non-constant variance model provided an adequate fit to the data for decreased hemoglobin; therefore, these data were not considered suitable for BMD modeling. BMD model prediction for decreased hematocrit and decreased erythrocyte count are shown in Tables A-6 and A-7, respectively. Of models meeting adequate fit criteria, the lowest BMDL_{1SD} values for each hematological end point were 0.13 mg/kg/day for decreased hematocrit (exponential 4 model; Figure A-4) and 0.12 mg/kg/day for decreased erythrocyte count (exponential 4 model; Figure A-5). Of these, the lowest BMDL_{1SD} of 0.12 mg/kg/day for decreased erythrocyte count was selected as the POD for derivation of the intermediate-duration oral MRL for 2,4-DNT. This value was divided by an uncertainty factor of 100 (10 for animals to human extrapolation and 10 for human variability) resulting in intermediate chronic-duration oral MRL of 0.001 mg/kg/day.

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Table A-6. Model Predictions for 2,4-DNT for Decreased Hematocrit (%) in Female Dogs Following 12 Months of Exposure (U.S. Army 1979)

Model	Test for significant difference p-value ^a	Variance p-value ^b	Means p-value ^b	Scaled residuals ^c			AIC	BMD _{1SD} (mg/kg-day)	BMDL _{1SD} (mg/kg-day)
				Dose below BMD	Dose above BMD	Overall largest			
Constant variance									
Exponential (model 2) ^d	0.01	0.34	0.06	-1.82	0.29	-1.82	78.08	6.77	4.09
Exponential (model 3) ^d	0.01	0.34	0.06	-1.82	0.29	-1.82	78.08	6.77	4.09
Exponential (model 4)^{d,e}	0.01	0.34	0.92	-0.07	0.04	-0.07	74.50	0.61	0.13
Exponential (model 5) ^d	0.01	0.34	0.92	-0.07	0.04	-0.07	74.50	0.61	0.13
Hill ^d	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linear ^f	0.01	0.34	0.06	-1.83	0.27	-1.83	78.15	6.96	4.31
Polynomial (2-degree) ^f	0.01	0.34	0.06	-1.83	0.27	-1.83	78.15	6.96	4.31
Polynomial (3-degree) ^f	0.01	0.34	0.06	-1.83	0.27	-1.83	78.15	6.96	4.31
Power ^d	0.01	0.34	0.06	-1.83	0.27	-1.83	78.15	6.96	4.31

^aValues >0.05 fail to meet conventional goodness-of-fit criteria.

^bValues <0.10 fail to meet conventional goodness-of-fit criteria.

^cScaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.

^dPower restricted to ≥1.

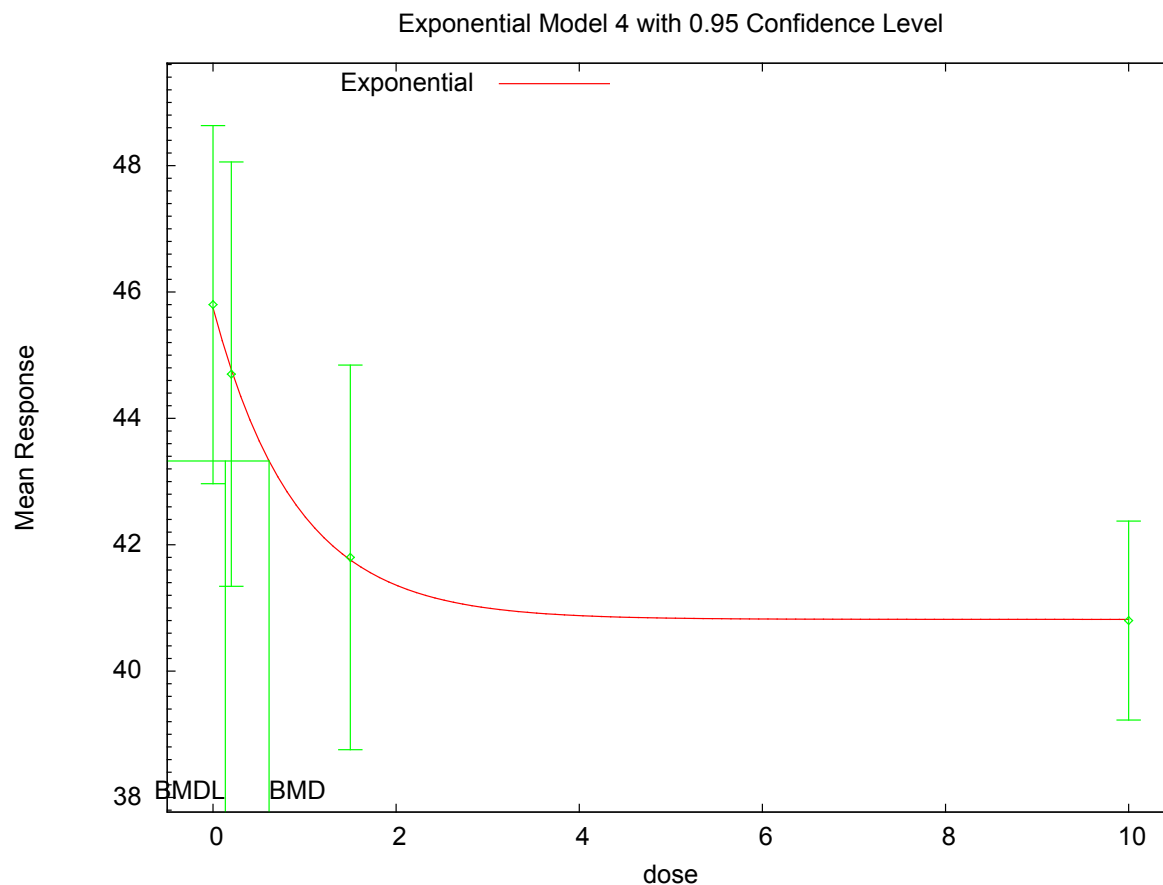
^eSelected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, the only models that provided adequate fit to the means were the Exponential 4 and 5 models (the Exponential 5 converged on to the Exponential 4).

^fCoefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., ₁₀ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

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Figure A-4. Fit of Exponential 4 Model to Data on 2,4-DNT for Decreased Hematocrit (%) in Female Dogs Following 12 Months of Exposure (U.S. Army 1979)



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Table A-7. Model Predictions for Decreased Erythrocyte Count in Dogs Treated with 2,4-DNT for 12 Months

Model	Test for significant difference p-value ^a	Variance p-value ^b	Means p-value ^b	Scaled residuals ^c			AIC	BMD _{1SD} (mg/kg-day)	BMDL _{1SD} (mg/kg-day)
				Dose below BMD	Dose above BMD	Overall largest			
Constant variance									
Exponential (model 2) ^d	0.0005	0.46	0.005	-2.40	0.43	-2.40	6.16	5.11	3.21
Exponential (model 3) ^d	0.0005	0.46	0.005	-2.40	0.43	-2.40	6.16	5.11	3.21
Exponential (model 4)^{d,e}	0.0005	0.46	0.91	-0.08	0.05	-0.08	-2.55	0.35	0.12
Exponential (model 5) ^d	0.0005	0.46	0.91	-0.08	0.05	-0.08	-2.55	0.35	0.12
Hill ^d	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linear ^f	0.0005	0.46	0.004	-2.41	0.35	-2.41	6.47	5.58	3.69
Polynomial (2-degree) ^f	0.0005	0.46	0.004	-2.41	0.35	-2.41	6.47	5.58	3.69
Polynomial (3-degree) [†]	0.0005	0.46	0.004	-2.41	0.35	-2.41	6.47	5.58	3.69
Power ^d	0.0005	0.46	0.004	-2.41	0.35	-2.41	6.47	5.58	3.69

^aValues >0.05 fail to meet conventional goodness-of-fit criteria.

^bValues <0.10 fail to meet conventional goodness-of-fit criteria.

^cScaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.

^dPower restricted to ≥1.

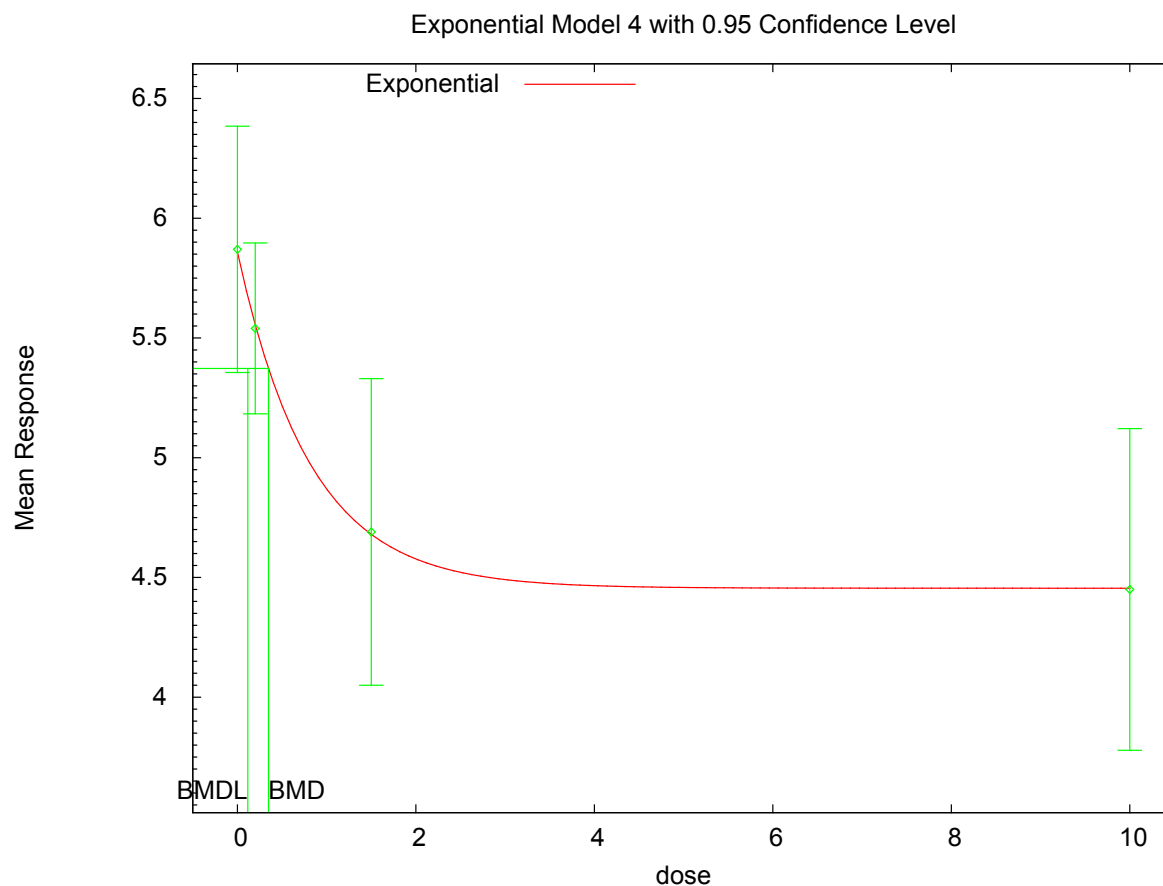
^eSelected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, the only models that provided adequate fit to the means were the Exponential 4 and 5 models (the Exponential 5 converged on to the Exponential 4).

^fCoefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., ₁₀ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

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Figure A-5. Fit of Exponential Model 4 to Data on Decreased Erythrocyte Count in Female Dogs Treated with 2,4-DNT for 12 Months



Uncertainty Factors used in MRL derivation:

- [] 10 for use of a LOAEL
- [X] 10 for extrapolation from animals to humans
- [X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: The hematological effects observed in this study are consistent with effects observed at higher doses in studies of intermediate duration (Hong et al. 1985; Lee et al. 1985; Kozuka et al. 1979; U.S. Army 1978b). Dogs were the most sensitive species in studies of chronic duration. In a 2-year study, male CD rats administered 2,4-DNT at 3.9 mg/kg/day showed decreased erythrocyte count after treatment for 12 months. Additional evidence for anemia, including further reductions in red blood cell count,

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decreased hematocrit, decreased hemoglobin, and a compensatory increase in reticulocytes, was observed in male and female rats administered high-dose 2,4,-DNT (34 and 45 mg/kg/day for males and females, respectively) for 12 or 18 months (Lee et al. 1985; U.S. Army 1979). CD-1 mice administered 2,4-DNT for 2 years showed no evidence of methemoglobin-induced anemia or compensatory reticulocytosis, except for decreased erythrocyte count and hemoglobin, and increased numbers of reticulocytes at the highest tested dose (898 mg/kg/day) (Lee et al. 1985; U.S. Army 1979).

Agency Contact (Chemical Manager): Carolyn Harper

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MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,6-DNT
CAS Numbers: 606-20-2
Date: October 2012
Profile Status: Final Draft for Pre-Public Comment
Route: ☐ Inhalation ☒ Oral
Duration: ☒ Acute ☐ Intermediate ☐ Chronic
Graph Key: 6
Species: Dog

Minimal Risk Level: 0.09 ☒ mg/kg/day ☐ ppm

References: U.S. Army. 1976. Mammalian toxicity of munitions compounds. Phase II: Effects of multiple doses. Part III: 2,6-Dinitrotoluene. Progress report no. 4. Fort Detrick, MD: U.S. Army, Medical Bioengineering Research and Development Laboratory. ADA062015.

Experimental design: Young beagle dogs (4 dogs/sex/group; age not specified) were administered 0, 4, 20, or 100 mg/kg 2,6-DNT in capsules for 13 weeks (U.S. Army 1976). Dogs were observed daily for behavioral changes and clinical signs of toxicity. Body weights were recorded weekly. Feed consumption was measured daily. Blood was taken before initiation of treatment and at 2, 4, 8, and 13 and/or 17 weeks (4-week post-treatment recovery period) for hematological (erythrocyte, reticulocyte, platelet, and total and differential leukocyte counts; Heinz bodies; hematocrit, hemoglobin, and methemoglobin concentrations; and mean cell volume, hemoglobin, and hemoglobin concentration) and clinical chemistry (glucose, urea nitrogen, levels of sodium, potassium, calcium, magnesium, and chloride; and serum enzyme activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) analyses. No additional assessments were conducted for acute-duration exposure. Assessments conducted for intermediate-duration exposure (4–13 weeks and 4-week post-treatment recovery period) included hematology and clinical chemistry; gross pathological examination, organ weights (heart, liver, spleen, kidneys, adrenals, and gonads), and microscopic examination of tissues (“various” tissues, not specified) were assessed in animals dying before the end of treatment, and at the end of the 13-week treatment period and the 4-week post-treatment recovery period. Bone marrow and kidney cultures were also maintained and cytogenetic analyses (of chromosome number and morphology) were performed.

Effects noted in study and corresponding doses: Acute-duration oral exposure of dogs to 2,6-DNT at ≥ 20 mg/kg/day show the development of anemia and compensatory hematopoiesis (U.S. Army 1976) (data summarized in Table A-8). Hematological effects of 2,6-DNT are initiated by methemoglobin production, which occurs when the ferrous iron in complex with the heme groups of hemoglobin is oxidized to ferric iron. Ferric iron does not bind oxygen, resulting in anemia. Ferric iron also contributes to the denaturation of hemoglobin and subsequent removal of erythrocytes from the blood. Heinz bodies (granules of denatured hemoglobin) are also detected within erythrocytes. Increased hematopoiesis is typically observed as a compensatory response to decreased erythrocyte count. Since immature erythrocytes are typically larger, mean cell volume and mean cell hemoglobin tend to be increased. Dogs treated at 20 mg/kg/day showed a statistically significant decrease in erythrocyte count and a significant increase in mean cell hemoglobin. At 100 mg/kg/day, more pronounced changes in these hematological parameters were observed; dogs showed statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and a statistically significant increase in reticulocyte count. In the low-dose group, similar hematological effects (decreased erythrocyte count, hemoglobin, and hematocrit, and increased reticulocytes) were observed, but these changes did not achieve statistical significance. Mid-

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and high-dose dogs (but not low-dose dogs) continued to show signs of anemia and compensatory hematopoiesis (decreased hematocrit and hemoglobin, and increased numbers of reticulocytes) for the duration of the 13-week study. Dogs treated at 20 mg/kg/day for 4 or 13 weeks and then removed from treatment showed recovery from hematological effects after 4 weeks; dogs treated at 100 mg/kg/day for 4 weeks did not show complete recovery until 19 weeks after cessation of treatment.

Table A-8. Hematological effects in Beagle Dogs Exposed to 2,6-DNT for 2 Weeks

End point	Dose (mg/kg/day)			
	0	4	20	100
Erythrocyte count (x10/mm)	5.62±0.16 (8) ^a	5.06±0.10 (8) [↓10]	4.73±0.20 (8) ^b [↓16]	1.85±0.28 (7) ^b [↓67]
Heinz bodies (%)	0.00±0.00 (8)	0.00±0.00 (8) [NA]	0.00±0.00 (8) [NA]	0.00±0.00 (7) [NA]
Reticulocytes (%)	0.76±0.07 (8)	1.10±0.13 (8) [↑45]	1.43±0.32 (8) [↑88]	16.99±3.33 (7) ^b [↑2,136]
Hematocrit (%)	42.1±1.7 (8)	38.9±0.6 (8) [↓8]	39.3±1.2 (8) [↓7]	22.9±2.8 (7) ^b [↓46]
Hemoglobin (%)	14.8±0.5 (8)	13.3±0.2 (8) [↓10]	13.0±0.4 (8) [↓12]	6.3±0.9 (7) ^b [↓57]
Methemoglobin (%)	0.0±0.0 (8)	0.0±0.0 (8) [NA]	0.0±0.0 (8) [NA]	0.0±0.0 (7) [NA]
Mean cell hemoglobin (micro µg)	26.3±0.2 (8)	26.3±0.3 (8) [0]	27.7±0.3 (8) ^b [↑5]	34.4±1.2 (7) ^b [↑31]

^aValues are means±standard error (number of animals) [percent change from controls].

^bStatistically significant based on analyses performed by the study authors (Dunnett's multiple comparison procedure).

DNT = dinitrotoluene; NA = not applicable

Source: U.S. Army 1976

Although incidence data were not reported, the study authors noted that at least three dogs (sex not specified) administered 2,6-DNT at 100 mg/kg/day showed clinical signs of toxicity (listlessness, incoordination, lack of balance, pale gums, dark urine, and weakness, particularly of the hind limbs) within the first 2 weeks of the study. One dog (a male) died during week 2. Similar (but milder) symptoms were reported in mid-dose dogs starting in week 4. No clinical signs of toxicity were observed in dogs administered the low dose of 2,6-DNT.

Histopathological assessments of tissues were not conducted in animals exposed for only 2 weeks. However, after 13-week of treatment, mild splenic hematopoiesis was noted in low-dose animals. Numerous lesions were detected in mid- and high-dose dogs; the number and severity of these lesions was increased at the high-dose. Affected organs included the liver (bile duct hyperplasia, degeneration, inflammation, and/or extramedullary hematopoiesis), kidney (degeneration and inflammation, dilated tubules), spleen (extramedullary hematopoiesis and lymphoid depletion), and testes (degeneration and atrophy of spermatogenic cells).

Dose and end point used for MRL derivation:

[] NOAEL [] LOAEL [X] BMDL 9.31 mg/kg was the BMDL_{1SD} for hematological effects (decreased erythrocyte count).

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Results of hematology assessments show that acute-duration, oral exposure of dogs to 2,6-DNT induced anemia and compensatory hematopoiesis (U.S. Army 1976). Statistically significant changes in hematological parameters, including decreased erythrocyte count and increased mean cell hemoglobin, were observed after treatment with 2,6-DNT at 20 mg/kg/day for 2 weeks (Table A-8). Changes to other hematological parameters only reached statistical significance at 100 mg/kg/day. Therefore, the most sensitive hematological parameters were erythrocyte count and mean cell hemoglobin. To determine the POD for derivation of the acute-duration oral MRL for 2,6-DNT, erythrocyte count and mean cell hemoglobin further evaluated by BMD analysis. All available continuous-variable models in the EPA BMDS (version 2.1) were fit to the data. The BMD and the 95% lower confidence limit (BMDL) were estimated for doses associated with a change of 1 standard deviation from the controls, and are in units of mg/kg-day. For continuous data, in the absence of a clear criteria as to what level of change should be considered adverse, the BMR is defined as a change equal to 1 standard deviation from the control mean (EPA 2000a). Adequate model fit is judged by three criteria: goodness-of-fit ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest benchmark dose (BMDL, the lower limit of a one-sided 95% confidence interval on the BMD) is selected as the POD when differences between the BMDLs estimated from these models are >3 -fold; otherwise, the BMDL from the model with the lowest AIC is chosen.

Neither the constant nor the non-constant variance model provided an adequate fit to the data for increased mean cell hemoglobin; therefore, these data were not considered suitable for BMD modeling. With non-constant variance model applied, the linear, polynomial, and power models provided an adequate fit to the data for decreased erythrocyte count (Table A-9). The polynomial and power models converged to the linear model. The figure shown from the linear model (Figure A-6) is representative of figures from the polynomial and power models (not shown). The BMDL_{1SD} value of 9.31 mg/kg/day derived from this model was selected as the POD. This value was divided by an uncertainty factor of 100 (10 for animals to human extrapolation and 10 for human variability) resulting in an acute-duration oral MRL of 0.09 mg/kg/day.

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Table A-9. Model Predictions for Decreased Erythrocyte Count in Dogs Treated with 2,6-DNT for 2 Weeks

Model	Test for significant difference p-value ^a	Variance p-value ^b	Means p-value ^b	Scaled residuals ^c			AIC	BMD _{1SD} (mg/kg-day)	BMDL _{1SD} (mg/kg-day)
				Dose below BMD	Dose above BMD	Overall largest			
Constant variance									
Linear ^d	<0.0001	0.08	0.25	-1.22	0.12	-1.22	-4.12	14.39	11.60
Non Constant variance									
Exponential (model 2) ^e	<0.0001	0.18	0.09	-1.55	1.26	-1.55	-3.38	8.15	6.00
Exponential (model 3) ^e	<0.0001	0.18	0.04	-1.60	0.64	-1.60	-1.90	11.15	6.17
Exponential (model 4) ^e	<0.0001	0.18	0.09	-1.55	1.26	-1.55	-3.38	8.15	5.84
Exponential (model 5) ^e	<0.0001	0.18	0.04	-1.60	0.64	-1.60	-1.90	11.15	6.07
Hill ^e	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linear^{d,f}	<0.0001	0.18	0.16	-1.42	0.13	-1.42	-4.47	12.21	9.31
Polynomial (2-degree) ^d	<0.0001	0.18	0.16	-1.42	0.13	-1.42	-4.47	12.21	9.31
Polynomial (3-degree) ^d	<0.0001	0.18	0.16	-1.42	0.13	-1.42	-4.47	12.21	9.31
Power ^e	<0.0001	0.18	0.16	-1.42	0.13	-1.42	-4.47	12.21	9.31

^aValues >0.05 fail to meet conventional goodness-of-fit criteria.

^bValues <0.10 fail to meet conventional goodness-of-fit criteria.

^cScaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.

^dCoefficients restricted to be negative.

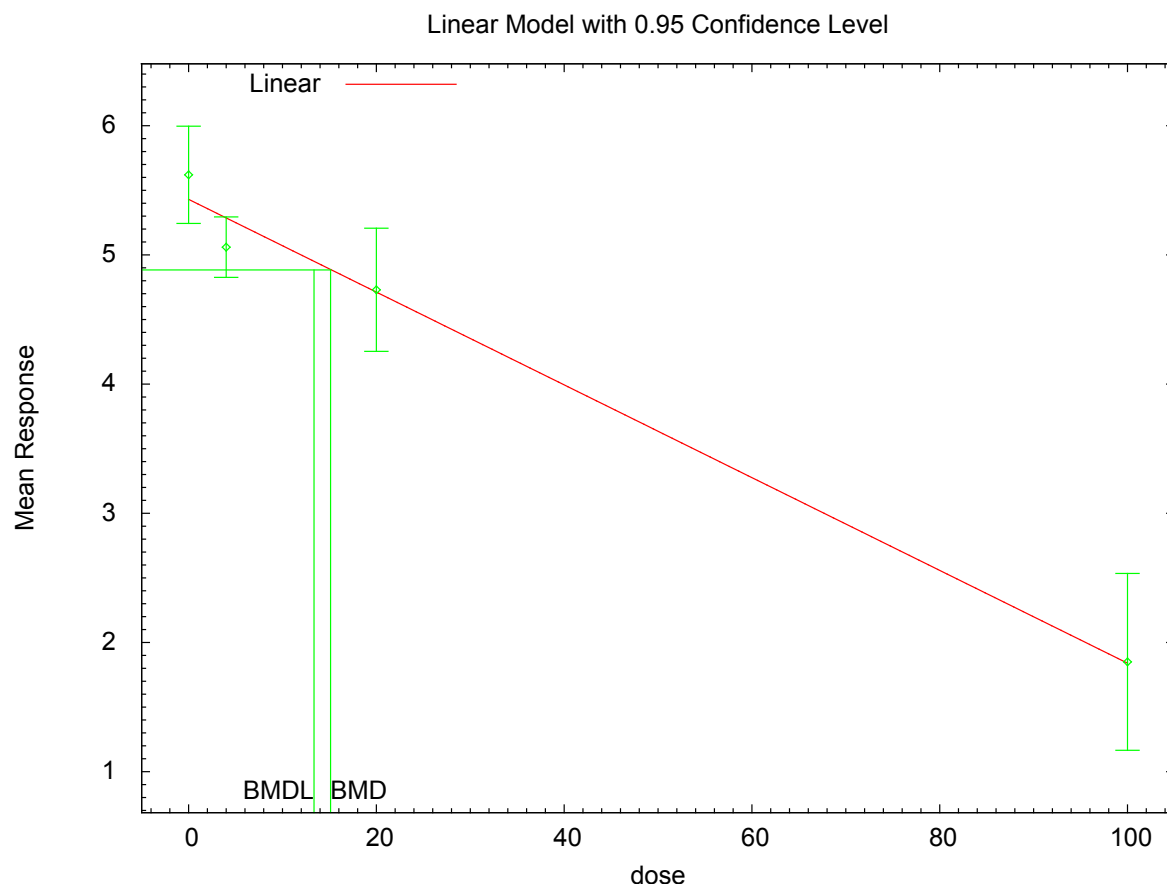
^ePower restricted to ≥1.

^fSelected model. Constant variance model did not provide adequate fit to variance data, but non-homogenous variance model did. With non-constant variance model applied, all models, except for the Exponential (means <0.1) and Hill models (computation failed), provided adequate fit to the means. The polynomial and power models all converged to the linear model.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., ₁₀ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; DNT = dinitrotoluene; NA = not applicable (BMDL computation failed); SD = standard deviation

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Figure A-6. Fit of Linear Model to Data on Decreased Erythrocyte Count in Dogs Treated with 2,6-DNT for 2 Weeks



BMDs and BMDLs indicated are associated with a change of 1 SD from the control, and are in units of mg/kg-day.

Source: U.S. Army 1976

Uncertainty Factors used in MRL derivation:

- [] 10 for use of a LOAEL
- [X] 10 for extrapolation from animals to humans
- [X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: Hematological effects consistent with methemoglobinemia-induced anemia and compensatory hematopoiesis have been

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observed in laboratory animals orally exposed to 2,6-DNT for acute and intermediate durations. No chronic-duration studies were identified that evaluated hematological effects after exposure to 2,6-DNT. Hematological effects (increased hemoglobin, hematocrit, and increased erythrocyte count, granulocyte, and reticulocyte counts) were observed in female Sprague-Dawley rats administered 2,6-DNT at 199 mg/kg/day via gavage for 48 hours (Deng et al. 2011). In intermediate-duration studies, dogs appear to more sensitive than rats or mice. In dogs orally exposed to 2,6-DNT at 4 mg/kg/day for 4 or 13 weeks, extramedullary erythropoiesis in the spleen secondary to methemoglobinemia and anemia was observed (U.S. Army 1976). Changes in hematological parameters associated with anemia and compensatory hematopoiesis (including decreased hematocrit and hemoglobin, and increased numbers of reticulocytes) occurred at 20 and 100 mg/kg/day. The incidence and severity of these effects were more pronounced at 100 mg/kg/day. Similar effects were observed in rats (U.S. Army 1976). In CD rats administered 2,6-DNT at ≥ 7 mg/kg/day and sacrificed after treatment for 4 or 13 weeks, increased incidences of extramedullary hematopoiesis and/or splenic hemosiderosis (increased iron accumulation) were observed. However, changes in hematological parameters (measured at 4, 8, and 13 weeks) indicative of anemia and compensatory hematopoiesis (including significant decreases in erythrocyte count, hematocrit, hemoglobin, and increased reticulocytes) were observed only at the highest tested dose (145 and 155 mg/kg/day for male and female rats, respectively); these effects were most pronounced after treatment for 4 weeks. Although histopathological effects (extramedullary hematopoiesis) was observed in CD-1 mice administered 2,6-DNT at ≥ 51 mg/kg/day (but not 11 mg/kg/day) for 4 or 13 weeks, no statistically significant changes in hematological parameters were seen. The study authors indicated that some blood samples clotted, making hematological analyses impossible to perform. The small number of animals evaluated likely contributed to the identification of histopathological findings of the spleen in the apparent absence of 2,6-DNT-induced hematological effects. The results of intermediate-duration studies indicate that hematological effects (or histopathological effects secondary to methemoglobinemia and anemia) are the most sensitive effects after exposure to 2,6-DNT; additional effects observed in intermediate-duration studies occurred at higher doses than hematological effects.

Agency Contact (Chemical Manager): Carolyn Harper

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MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2,6-DNT
CAS Numbers: 606-20-2
Date: October 2012
Profile Status: Final Draft for Pre-Public Comment
Route: ☐ Inhalation ☒ Oral
Duration: ☐ Acute ☒ Intermediate ☐ Chronic
Graph Key: 12
Species: Dog

Minimal Risk Level: 0.004 ☒ mg/kg/day ☐ ppm

Reference: U.S. Army. 1976. Mammalian toxicity of munitions compounds. Phase II: Effects of multiple doses. Part III: 2,6-Dinitrotoluene. Progress report no. 4. Fort Detrick, MD: U.S. Army, Medical Bioengineering Research and Development Laboratory. ADA062015

Experimental design: Young beagle dogs (4 dogs/sex/group; age not specified) were administered 0, 4, 20, or 100 mg/kg 2,6-DNT in capsules for 13 weeks (U.S. Army 1976). Dogs were observed daily for behavioral changes and clinical signs of toxicity. Body weights were recorded weekly. Feed consumption was measured daily. Blood was taken before initiation of treatment and at 2, 4, 8, and 13 and/or 17 weeks (4-week post-treatment recovery period) for hematological (erythrocyte, reticulocyte, platelet, and total and differential leukocyte counts; Heinz bodies; hematocrit, hemoglobin, and methemoglobin concentrations; and mean cell volume, hemoglobin, and hemoglobin concentration) and clinical chemistry (glucose, urea nitrogen, levels of sodium, potassium, calcium, magnesium, and chloride; and serum enzyme activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) analyses. Animals (1 sex/group) were sacrificed after 4 or 13 weeks of continuous treatment; an additional dog/sex/group was discontinued treatment after 4 or 13 weeks and sacrificed after 4 weeks of recovery (week 8 or 17) to evaluate the reversibility of effects. The two high-dose dogs removed from treatment at 4 weeks were not sacrificed until 19 weeks after cessation of treatment to test the reversibility of effects after a longer recovery period. When animals were moribund or at study termination, they were examined for gross lesions; major organs and tissues were weighed (heart, liver, spleen, kidneys, adrenals, and gonads); and "various" tissues (number not specified) were subjected to histopathological examinations. Bone marrow and kidney cultures were also maintained and cytogenetic analyses (of chromosome number and morphology) were performed.

Effects noted in study and corresponding doses: Intermediate-duration oral exposure of dogs to 2,6-DNT at 4 mg/kg/day produced extramedullary erythropoiesis (formation of erythrocytes outside of the bone marrow) in the spleen secondary to methemoglobinemia and anemia (U.S. Army 1976). Hematological effects and compensatory erythropoiesis induced by 2,6-DNT are initiated by methemoglobin production, which occurs when the ferrous iron in complex with the heme groups of hemoglobin is oxidized to ferric iron. Ferric iron does not bind oxygen, resulting in anemia. Ferric iron also contributes to the denaturation of hemoglobin and subsequent removal of erythrocytes from the blood. Increased erythropoiesis is typically observed as a compensatory response to decreased erythrocyte count.

Mortality occurred in dogs administered 20 and 100 mg/kg/day. Two mid-dose female dogs died in week 9; all high-dogs died by week 8. Effects observed in dogs treated at 20 and 100 mg/kg/day were clinical signs of neurotoxicity (listlessness, incoordination, and lack of balance), decreased feed consumption and subsequent reductions in body weight, and changes in hematological parameters associated with anemia and compensatory hematopoiesis (including decreased hematocrit and

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hemoglobin, and increased numbers of reticulocytes). The incidence and severity of these effects were more pronounced at 100 mg/kg/day relative to 20 mg/kg/day. Dogs administered 2,6-DNT at 4 mg/kg/day showed no clinical signs of toxicity, and although similar hematological effects occurred, these changes were not statistically significant. No significant effects on clinical chemistry end points were observed in any 2,6-DNT treatment group. In general, dogs treated at 20 mg/kg/day for 4 or 13 weeks and then removed from treatment showed recovery from neurotoxicity and hematological effects after 4 weeks; dogs treated at 100 mg/kg/day for 4 weeks did not show complete recovery until 19 weeks after cessation of treatment.

Histopathological evaluation of the spleen showed an increased incidence of extramedullary erythropoiesis, an adaptive response to 2,6-DNT-induced methemoglobinemia and anemia, in dogs administered ≥ 4 mg/kg/day for 4 or 13 weeks (Table A-10). The incidence and severity of this lesion was dose-related. Additional histopathological changes observed dogs administered 2,6-DNT at 20 or 100 mg/kg/day for 4 or 13 weeks included effects on the thymus (involution), liver (extramedullary hematopoiesis, bile duct hyperplasia, degeneration, and inflammation), kidneys (degeneration, inflammation, and dilated tubules), and testes (degeneration and/or decreased spermatogenesis). High-dose dogs also showed evidence of lymphoid depletion in the spleen and lymph nodes. No other treatment-related histopathological changes were observed in dogs dosed with 2,6-DNT at 4 mg/kg/day for 13 weeks.

Table A-10. Extramedullary Erythropoiesis of the Spleen in Beagle Dogs Exposed to 2,6-DNT for 4 or 13 Weeks

Timepoint (weeks)	Dose (mg/kg/day)			
	0	4	20	100
4	0/2 ^a	1/2 (mild)	2/2 (moderate)	2/2 (1 mild; 1 markedly severe)
13	0/2	2/2 (1 minimal; 1 mild)	3/3 (1 minimal, 1 mild, 1 moderate)	4/4 (2 marked, 2 markedly severe)

^aNumber examined/number affected (severity of lesion).

DNT = dinitrotoluene

Source: U.S. Army 1976

Dose and end point used for MRL derivation:

[] NOAEL [X] LOAEL 4 mg/kg for mild extramedullary erythropoiesis in the spleen.

The LOAEL value of 4 mg/kg/day for an increased incidence of extramedullary erythropoiesis in the spleens of dogs was identified as the POD for derivation of the intermediate-duration oral MRL for 2,6-DNT (U.S. Army 1976). Histopathology data were not suitable for BMD modeling, since the number of animals evaluated at each dose and time was small (n=2 animals). Therefore, the LOAEL value for 4 mg/kg/day was used at the POD. This value was divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability), resulting in an acute-duration oral MRL for 2,6-DNT of 0.004 mg/kg/day.

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Uncertainty Factors used in MRL derivation:

- [X] 10 for use of a LOAEL
- [X] 10 for extrapolation from animals to humans
- [X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information which lend support to this MRL: Similar histopathological effects (extramedullary hematopoiesis and/or splenic hemosiderosis), indicative of an adaptive response to anemia and compensatory erythropoiesis, were observed in CD rats administered 2,6-DNT at ≥ 7 mg/kg/day and in CD-1 mice administered 2,6-DNT at ≥ 51 mg/kg/day (but not 11 mg/kg/day) for 4 or 13 weeks (U.S. Army 1976). Hematological effects were also identified in intermediate-duration studies. In these studies, dogs appear to be the most sensitive species. Dogs treated at 20 mg/kg/day showed a statistically significant decrease in erythrocyte count and a significant increase in mean cell hemoglobin at 2 weeks. At 100 mg/kg/day, more pronounced changes in these hematological parameters were observed; statistically significant reductions in erythrocyte count, hematocrit, and hemoglobin and a statistically significant increase in reticulocyte count were observed. In CD rats, changes in hematological parameters indicative of anemia (significant decreases in erythrocyte count, hematocrit, and hemoglobin) and compensatory hematopoiesis (increased reticulocytes) were observed only at the highest tested dose (145 and 155 mg/kg/day for male and female rats, respectively). Although histopathological effects (extramedullary hematopoiesis) was observed in CD-1 mice administered 2,6-DNT at 51 mg/kg/day (but not 11 mg/kg/day), no statistically significant changes in hematological parameters were seen after treatment for 4 or 13 weeks. The study authors indicated that some blood samples clotted, making hematological analyses impossible to perform. The small number of animals evaluated likely contributed to the identification of histopathological findings of the spleen in the apparent absence of 2,6-DNT-induced hematological effects.

Agency Contact (Chemical Manager): Carolyn Harper

APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

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

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

Chapter 2

Relevance to Public Health

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

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

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

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

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived 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.

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MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

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

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (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 levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures 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, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper-bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

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LEGEND**See Sample LSE Table 3-1 (page B-6)**

- (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. Typically when sufficient data exist, 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 Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.
- (2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.
- (3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).
- (4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).
- (5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.
- (6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to "Chemical x" via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).
- (7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.
- (8) NOAEL. A 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").

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- (9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.
- (10) Reference. The complete reference citation is given in Chapter 9 of the profile.
- (11) CEL. A 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 that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Sample Figure 3-1 (page B-7)

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 acute and intermediate 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/m³ or ppm and oral exposure is reported in mg/kg/day.
- (16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. 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 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

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

SAMPLE

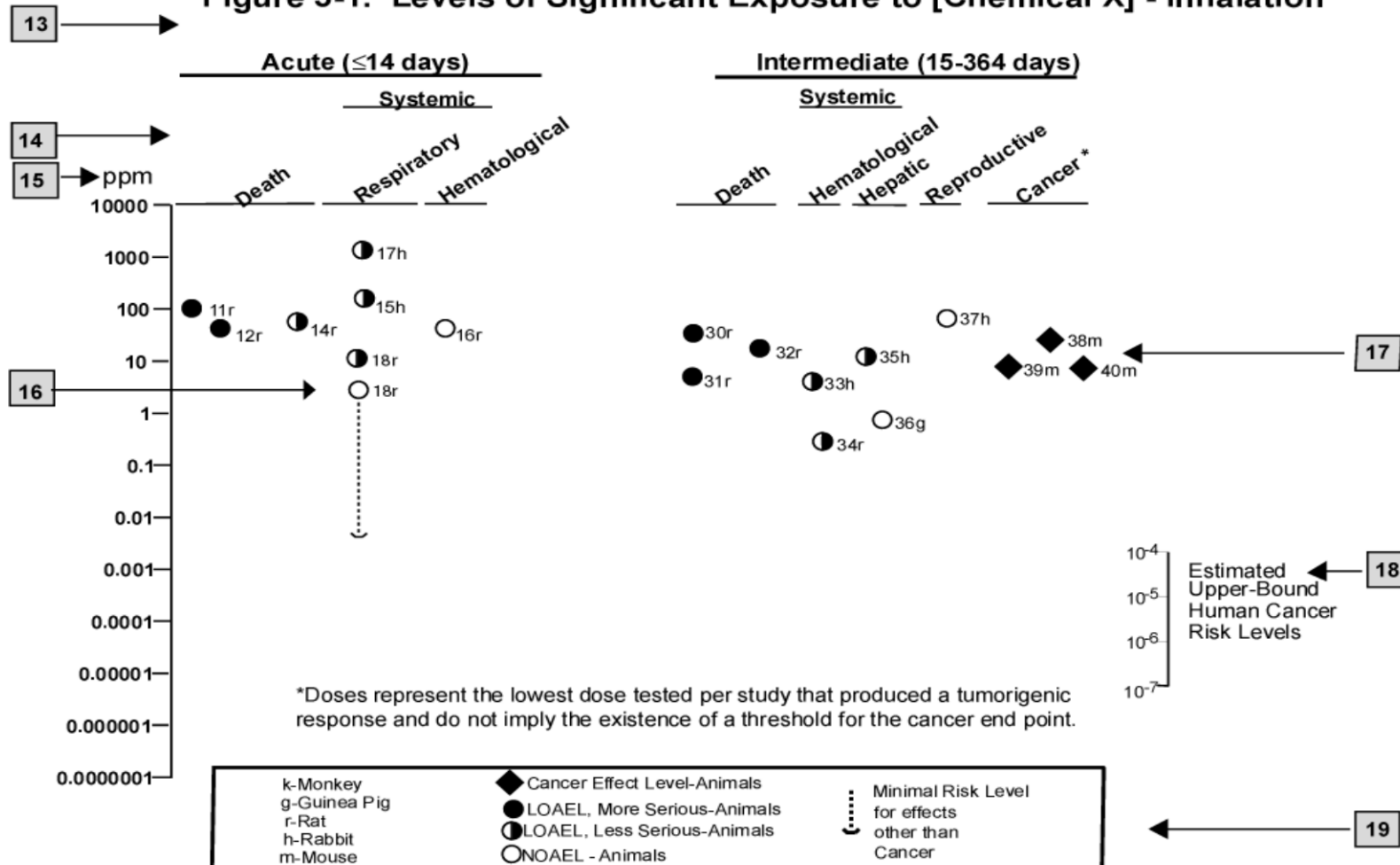
1 →

Table 3-1. 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 5 d/wk 6 hr/d	Resp	3 ^b	10 (hyperplasia)		Nitschke et al. 1981
	CHRONIC EXPOSURE							
	Cancer						11	
						↓		
	38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
	39	Rat	89–104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
	40	Mouse	79–103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982
12 →	^a The number corresponds to entries in Figure 3-1. ^b Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5x10 ⁻³ ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).							

SAMPLE

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



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APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BMD/C	benchmark dose or benchmark concentration
BMD _x	dose that produces a X% change in response rate of an adverse effect
BMDL _x	95% lower confidence limit on the BMD _x
BMDS	Benchmark Dose Software
BMR	benchmark response
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor

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DOT	Department of Transportation
DOT/UN/	Department of Transportation/United Nations/
NA/IMDG	North America/Intergovernmental Maritime Dangerous Goods Code
DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F ₁	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
K _d	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 ₅₀	lethal concentration, 50% kill
LC _{Lo}	lethal concentration, low
LD ₅₀	lethal dose, 50% kill
LD _{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	lutinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT ₅₀	lethal time, 50% kill
m	meter
MA	<i>trans,trans</i> -muconic acid
MAL	maximum allowable level
mCi	millicurie

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

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OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD ₅₀	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

APPENDIX C

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

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